CHAPTER 2

Planning

PRE-PROJECT PLANNING CONSIDERATIONS: IMPORTANCE OF GENETIC DIVERSITY IN SEAGRASS POPULATIONS

As habitat loss, fragmentation and geographic isolation of relict habitat has increased worldwide, scientists and resource managers, have become justifiably alarmed at the rapid loss of species and genetic diversity within remaining populations. Seagrass beds are no exception (*sensu* Ruckelshaus 1994a, Williams et al. 1996). In recent years the genetic status of seagrass beds has begun to be examined and the impact of human encroachment on its genetic diversity questioned (Alberte 1993, Williams and Davis 1993). However, as is the case in most wild populations, quantitative information regarding the genetics of individual plants, let alone populations, is scanty; and management decisions at the population level are those that most resource managers are likely to make. The question remains, however, does reduced genetic diversity actually matter in terms of population recovery trajectories and, thus, persistence of seagrass populations? Moreover, do differences in genetic diversity among planted and natural beds signal the disintegration of gene complexes specifically adapted to local conditions (S. Williams, Univ. California, San Diego, CA., pers. com.)? Because quantitative phylogenetic analysis of seagrasses is only beginning (Procaccini and Mazzella 1996, Waycott and Les 1996), it is currently impossible to infer adaptive value for any attributes of seagrasses, including gene complexes. Genetic diversity is generally assumed to be crit-

ical to the survival of restored populations, but before genetic screening can become a management tool, much more research is needed to clarify the consequences of any changes in genetic structure of seagrass populations as the result of habitat destruction and planting projects.

Some information is available regarding the role of genetic vs. environmental controls of seagrass. Backman (1991) concluded that genetic variation accounted for 14 percent of morphologic variation, environmental setting 32 percent, and interaction of genetic and environmental factors 35 percent. Based on these findings, Backman (1991) also suggested differentiating *Z. marina* into five varieties. However, employing similar techniques, Dennison and Alberte (1986) conducted reciprocal transplants of a *Z. marina* population in Massachusetts and found that growth responses were largely environmental and not genetic.

But for management purposes, maintenance of seagrass populations must be based on more than correlative inference of adaptive capabilities. Questions regarding population maintenance and genetic structure therefore must include some comprehension of how connected seagrass populations are along coastal areas. This includes some assessment of gene flow, genetic drift, influence of founder effects, existence of heterozygote advantages, identification of selection pressures, and determining the existence of metapopulations (*sensu* Orth et al. 1994).

The few extant publications differ somewhat in their conclusions regarding genetic variation in seagrass beds. Laushman (1993) found that genetic variance of *Zostera* populations was less within bays than among bays. Alberte et al. (1994) asked a different question and that is how genetic relatedness is associated with geographic separation; like most spatially dependent data, they found that the closer the eelgrass patches were to each other the more alike they were. Ruckelshaus (1994b) suggested that the role of local extinction as the result of disturbance (e.g., sedimentation, storm-induced scour) and subsequent recolonization of such areas may be an important source of genetic diversity. Because spatial and temporal variation in disturbance have long been known to have dramatic impacts on genetic diversity (e.g., founder effect and subsequent genetic drift: Futuyma 1986), historical contingency is often the initial basis for differences in genetic makeup among geographically isolated populations. Without some idea of the historical context of a population, however, it may be difficult to determine what management strategy would best serve as a response to detection of lowered genetic diversity, especially in anthropogenically-disturbed areas.

These population level questions are difficult to answer even in terrestrial environments where direct observation is much easier than in seagrass beds. For example, gene flow among geographically-separate populations is generally considered to be significant if only one individual exchanges genetic information with another individual in a separate population once each generation. In the case of clonal plants such as seagrass, it is not clear what a generation time might be. An individual ramet can live for days (e.g., *Halophila* spp.) or years (e.g., *Thalassia*). However it reproduces both by seed which is the result of genetic recombination (which can incorporate genetic information from an individual from another population: gene flow); and by vegetative branching which involves no external genetic input and merely produces a second ramet that has the same genetic makeup as its first (i.e., a clone, excluding potential somatic mutations). Therefore, in a genetic sense, populations that employ a consistent, season-to-season and year-to-year component of vegetative (i.e., asexual) reproduction, a single generation may be composed of changing individual ramets but one genetic constitution. Thus, a single generation could conceivably last for decades or longer and for these populations, exchange of genetic material (gene flow) might not be needed among geographically isolated populations for many years. However, we do not know the appropriate time scales over which to evaluate the relationship between genetic diversity and gene flow, one reason being because we do not know how long a generation is for these clonal plants. On the other hand, most evidence points to interdigitation of genets on small (< 1 m) spatial scales, thus gene flow should not be limited across small spatial scales (Ruckelshaus 1995). Local gradients in genetic structure might then be assumed to be the product of locally abrupt selection gradients (i.e., water depth and light availability, *sensu* Fain et al. 1992).

It is also difficult to determine the size of a population that is interbreeding (effective population size; but see Ruckelshaus 1994b). In seagrass beds, Orth et al. (1994) reported that seeds typically disperse near their source although many avenues for long distance dispersal exist, but others have found evidence for both long- and short-distance dispersal (Ruckelshaus 1995). Once a location is colonized (or re-colonized) the degree of subsequent isolation raises questions of founder effects (the initial reduction in local genetic diversity given that the few founders do not represent the genetic richness of the parent population) which can result in locally distinct genetic structure in a group of plants. A similar scenario of limited gene flow was inferred by Alberte et al. (1994), a phenomenon that supports the metapopulation theory of Orth et al. (1994). Subsequent long-term reproductive isolation can also enhance genetic differences among groups, especially in the aftermath of a founder effect. Another means of enhancing local genetic diversity was described by

Ruckelshaus (1994b) where *Zostera* presence in an area of the Pacific Northwest experienced repeated local extinctions and recolonization which enhanced genetic diversity.

Other potential (though not insurmountable) problems exist in the utilization of these data; our knowledge of the appropriate genetic indicators is limited, which imposes potentially severe technological limitations. Estimations of population size using genetic probes typically requires that the alleles being targeted are not acted on by natural selection. Because we do not know what aspects of a seagrass plant's biology are influenced by a given allele, it is conceivable that natural selection could be simultaneously altering allele frequencies being tested and, thus, biasing our interpretation of recent selection events. This is particularly a controversy with allozyme techniques (Futuyma 1986; p. 98), although these problems can be resolved if appropriate testing of probes is conducted and reported (S. Williams, Biology Dept., San Diego State Univ., San Diego, CA., M. Ruckelshaus, National Marine Fisheries Service, Seattle, WA., pers. com.).

The point here is that without knowledge of how often genes are exchanged among populations, the boundaries of populations, the existence or role of metapopulations, the duration of a generation, and the importance of seeds (a product of genetic recombination) in maintaining populations (and for the moment ignoring mutation), it is very difficult to direct specific management actions (i.e., site-specific) based on static surveys of genetic diversity. However, some guidance can be given even with preliminary data. If genetic variation were found to be partitioned more among sites of known geographic separation (therefore, populations are loosely defined as different sampling sites), rather than within sites, it would be advisable to equally protect geographically isolated sites (populations), and treat them as a larger, continuous resource rather then self-sufficient, isolated populations.

Following the findings of Ruckelshaus (1994a,b), we suggest that the environmental and geographic context under which surveys of genetic structure and diversity are undertaken are as relevant as the detailed information regarding polymorphic loci that emerge from any given study (*sensu* Ruckelshaus 1994b). For example, eelgrass beds on the West Coast of the United States exist in a geographically fragmented distribution among various water bodies and have, in the last century, experienced high localized losses. The situation on the East Coast is somewhat different. Although localized losses have occurred on the East Coast too, *Z. marina* populations went through a bottleneck with the wasting disease of the 1930's. These historical contingencies represent events known to affect the genetic structure of populations (geographic isolation and bottlenecks). It may be that the lack of histori-

cal information on many of these populations and the absence of data on effective population size and gene flow (particularly human-mediated gene flow such as seed transport, may severely bias generalizations regarding erosion of genetic diversity). In the Pacific Northwest, such a process was an effective mechanism for the introduction of an entire species, *Zostera japonica*, in the last quarter century.

It is critical to separate naturally low genetic diversity from an anthropogenically-imposed loss of diversity when attempting to set management standards for protection and maintenance of genetic structure (i.e., one may be setting baseline conditions too low). Another reason for differentiating naturally-low genetic diversity from human-induced declines is that some clonal plants with low genetic diversity can persist for millennia (Cook 1985). Laushman (1993) suggests that hydrophilic taxa (including seagrass) have intrinsically lower heterozygosity, polymorphic loci and alleles per locus than non-hydrophytes, meaning that simple identification of low genetic diversity may not signal a management dilemma (but see Alberte et al. 1994). Thus, it is the detection of the erosion of genetic structure, a measure that requires evaluation over appropriate temporal and spatial scales, that will determine the response by management (*sensu* S. Williams, Biology Dept., San Diego State Univ., San Diego, CA., pers. com.).

Even if genetic differences are seen among populations we still do not know if those differences are important to maintaining seagrass populations in areas under existing conditions. However, Alberte (1993) has found preliminary evidence of reduced genetic diversity of *Zostera* in impacted, as opposed to unimpacted, areas. Similarly, Williams and Davis (1993) have found evidence for reductions in counts of polymorphic loci among recently transplanted beds when compared to older, more persistent beds in San Diego Bay from which the transplants originated. More Williams et al. (1996) demonstrated "...that genetic diversity (percentage of polymorphic loci, allele richness, expected and observed heterozygosity, and proportion of genetically unique individuals) was significantly reduced in transplanted eelgrass beds." Williams et al. (1996) attributed this reduction in genetic diversity to small sizes of planting projects, limited geographic range in donor stock collection, and failed sexual reproduction, all inferring founder effects. Geographic variability has since been shown to contribute greatly to differences in genetic composition among planted and natural beds (Williams and Orth 1998). This is a potentially serious trend, even though very old (non-seagrass) plant clones of low genetic diversity have been shown to exist in nature. The response of decreased genetic diversity in recently transplanted beds observed by Williams and her co-workers (1996) is exactly what might be expected of a disintegrating population; one potentially becoming less able to respond to the vagaries of environmental variation produced by the comparative-

ly rapid human alteration (as opposed to evolutionary time) of the nearshore environment. Localized extinctions might be expected under these conditions. However, reduced genetic diversity in transplanting might also be expected as it could mimic a natural founder effect, and with time, genetic diversity may increase in these planted beds especially as sexual reproduction (seeding) contributes more to these beds over time. Thus, it is possible that the small-scale variation in genetic structure observed in natural eelgrass beds (Williams et al. 1996) is the result not only of tillering and branching, but deposition of seeds (the product of genetic recombination). Thus, we are not yet sure whether reduced genetic structure and diversity are long-term problems. If these planted beds are genetically deficient compared to their parent beds, they may certainly pose short-term problems if managers rely on planted beds as donor stock areas to mitigate for losses of natural beds. Planted beds may not have the genetic makeup to deal with stressed conditions. Basic research on the phylogenetics of seagrasses is greatly needed. Recent advances by Waycott and Les (1996) and Uchiyama (1996) provides guidance on the comparative status of breeding systems which in turn allows us to begin to understand the role of vegetative vs. sexual reproduction in species' maintenance (*sensu* Procaccini and Mazzella 1996).

All of the above questions aim at resolving the issue as to whether differences in genetic diversity, or even its loss, means anything to the short- or long-term survival of seagrass populations under planting operations? Do changes in diversity influence maintenance of increasingly impacted and fragmented seagrass habitats? How long do these differences persist? Can we collect information on genetic structure over spatial and temporal ranges and with sufficient resolution to formulate management directives? Without specific, continued funding to support this work, the answer is unfortunately, "no." Therefore the question remains as to whether seagrass populations, particularly those currently becoming fragmented, will have the resilience to deal with the environmental changes brought on by human encroachment in the coastal zone. It seems to be an extraordinarily risky gamble to assume that present rates of habitat loss do not constitute a threat to the genetically-based resilience of some seagrass populations. We concur with Alberte et al. (1994) that "Studies that examine genetic structure of populations over time in disturbed and undisturbed habitats are needed so that the impacts of chronic habitat deterioration on genetic stability and resilience of (*Zostera marina*) can be ascertained." We recommend that scientists and managers continue to investigate and take seriously the threat of diminished genetic diversity and population isolation (especially when combined with degradation of environmental conditions critical for seagrass growth, such as light) that is anthropogenically imposed (*sensu* Williams et al. 1996). Thus, conservation of existing stocks and avoidance of population fragmentation and isolation would be a rational approach until a decision process based on data is available.

At present it is our opinion that except for highly impacted estuaries, the major short-term problems of maintaining seagrass beds will be providing suitable water clarity, appropriate nutrient levels (which also influence water clarity), and minimizing direct physical disturbance (e.g., dredging). Problems of restoring seagrass beds are largely ones of appropriate site selection and subsequent bioturbation. No gene complex can provide protection against grossly insufficient light, excessive nutrient loading, or the depredations of bioturbating organisms in a recently planted bed. Following Williams et al. (1996), we suggest that in practice, interim concerns regarding genetic diversity should be met by selecting planting stock from beds throughout the water body which is closely connected with the planting site. Stock selection (dealt with more completely, below) thus follows recommendations very similar to that of Addy (1947), especially in light of the fact that Fain et al. (1992) and Ruckelshaus (1994b) have both found greater genetic similarity within seagrass populations at similar tidal elevations than among populations. Any proposals to conduct surveys of genetic diversity of seagrass beds must contain provisions for periodic resampling to assess the influence of periodic disturbance and seedling colonization (Laushman 1993, Ruckelshaus 1994b) on genetic makeup, realizing of course that such sampling may have to be conducted for many years to detect the temporal sequence of disturbance that actually influences local genetic diversity (*sensu* Ruckelshaus 1994b).

More Pre-Project Planning Considerations: Seagrass Bed Spatial Requirements and Planting Site Surveys

Acquiring pre-impact data on seagrass distribution and environmental conditions at a site is vital to good planning, but there are constraints in obtaining those kinds of data. One problem is that site surveys are often done at a single point in time. The problems with one-time surveys of impacted sites have been manifested in many ways. One way is when decisions are made to place channels among patchy seagrass beds. Typically, a survey such as an aerial photograph will be employed to pick the alignment that will minimize impacts to present-day seagrass patches. However, because seagrass beds are spatially dynamic in time (i.e., they move: Orth 1977, Marba et al. 1994, Marba and Duarte 1995), such an alignment will almost certainly result in a decision to effect a long-term reduction in seagrass abundance because seagrass patches require that today's unvegetated space be available for them to occupy in the future (Figure 1.2). Thus, if a portion of the unvegetated space is removed from among patchy seagrass beds, it is unavailable for colonization. Therefore, when the space occupied by present-day seagrass is vacated (via bed migration or mortali-

ty), there will be insufficient space for colonization and a local net decrease in over-all seagrass abundance, *even though no seagrass was immediately impacted* (*sensu*, Figure 1.2). This is the kind of impact that has been mitigated for in the past (Short 1993).

To demonstrate this effect, we have plotted the cumulative amount of bottom area near Beaufort, North Carolina, covered by seagrass in several 50 x 50 m plots which we have monitored for almost four and one-half years (Figure 1.2). These plots were mapped repeatedly for the presence/absence of seagrass cover with 1 m^2 resolution. Thus, over time, we counted the number of new square meters of bottom space occupied by seagrass each subsequent survey time, but excluding any m^2 locations that had ever had seagrass since the initial survey time (i.e., once a m^2 location is scored as having seagrass, it will never be added to this cumulative value again in the study period; only locations that had not been previously observed to have seagrass can be added). An important point is that the average percent cover at each site was very stable over the survey period. What this graph reveals is that sites that have nearly 100 percent cover to begin with, of course, remain at that level over time. However, sites that have lower percent coverage (y-intercept value)at any one point in time (here in North Carolina, largely the result of wave and tidal current effects), have had seagrass occupy twice the number of m^2 areas in that 4.5 year period than were observed at any one point in time. This cumulative coverage (over time) represents the spatial requirements needed to maintain a representative seagrass bed in a given physical setting over time; here at least twice the area of the standing seagrass coverage is required to sustain the patchy seagrass cover over a 4.5 year period.

With enough time, one would expect that ultimately all possible locations would eventually have supported seagrass cover; this is evidenced by the "hih2" site where cumulative coverage was still increasing at the May 1995 survey time. The asymptote of the cumulative coverage lines results at least in part for the tendency for m^2 areas near to existing patches of seagrass to be lost and recolonized more frequently than m^2 areas distant from a patch. What this graph clarifies is that a decision to remove a portion of the unvegetated space among seagrass patches through conversion to a channel or some non-seagrass habitat will, in many instances, result in the additional loss of seagrass acreage within a four-year period. Also, we hypothesize that depending on the alignment of a channel and the direction of bed migration, the channel may act as an interceptor, creating a large zone of low seagrass abundance in the down-migration direction, much as a snow fence or sand jetty accumulates material leaving the down-flow direction starved for that material. Thus, a knowledge of the spatial dynamics of seagrass beds over time is critical to maintaining present-day levels of seagrass acreage, information that is probably critical for the *Halophila* genera in particular.

PLANNING FOR EXECUTION OF A PLANTING PROJECT

In order to prepare for a seagrass planting project, several factors must be considered. As in Fonseca (1994), the heading for each sub-section below can serve as an abbreviated checklist of information needs and subsequent actions which should be anticipated. Common to all these considerations is the need for early coordination with State and federal resource agencies. Since many States have a management system set up for federal agency review of such plans, early coordination can resolve regulatory problems before they become costly. In addition, we present a decision flow diagram (Figure 2.1) as a summary of this section. We suggest that readers familiarize themselves with this section before attempting to apply the decision process in Figure 2.1. When needed, they should also consider the guidance on how elevation affects seagrass survival through the interaction of tides and light regime presented by Dennison and Kirkman (1996; see section on Emersion Effects, below).

IDENTIFICATION OF PROJECT GOALS

Although there are many methods to plant seagrass, frequently the goals of a project are not defined. Is the project for restoration or compensatory mitigation? Although the differences in project goals have little to do with the execution of the planting technique, it is important to recognize that planting in exchange for permitted losses may elicit different responses from resource agencies than planting for the sake of restoration only. For a review which touches on agency concerns, the reader should refer to Fonseca (1989a, 1992, 1994). Project goals should identify the species of plants that are to be used. Eventually attaining the same seagrass species as what was lost with an equal area of bottom covered is a logical, ecologically defensible goal.

PRE-CONSTRUCTION PLANNING

Because population growth rate varies with geographic location (and planting spacing), the timetable for meeting project goals will vary. For example, at ~ 1 m spacing, it will typically require two years to reach coalescence of planted areas for a shoalgrass bed in the Florida panhandle whereas it may take only six months to reach the same level of coverage for the same species in the Florida Keys (Fonseca et al. 1987c). This time lag should be anticipated for any planting and varies both by ecoregion and initial spacing of plantings (Fonseca et al. 1996a).
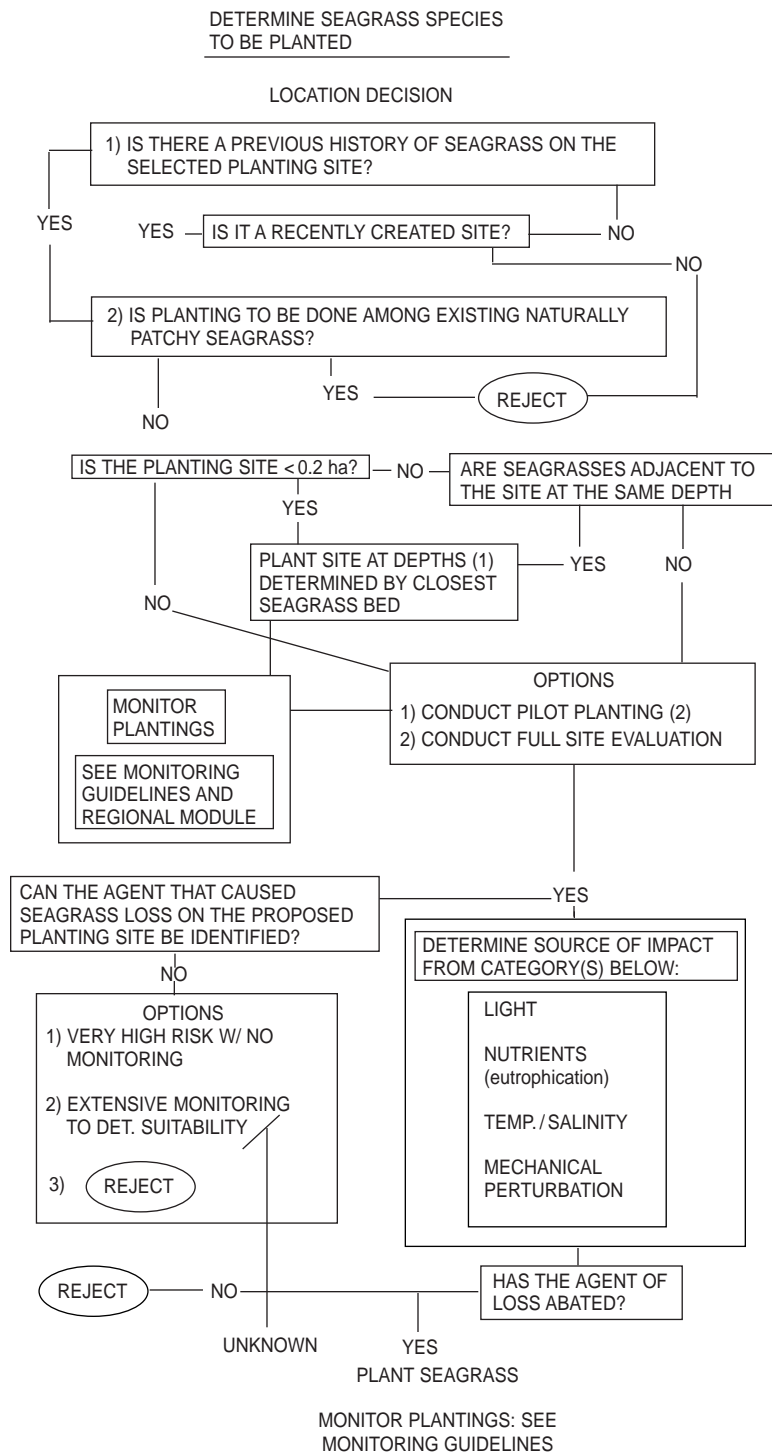
DETERMINE SEAGRASS SPECIES
TO BE PLANTED

LOCATION DECISION

1) IS THERE A PREVIOUS HISTORY OF SEAGRASS ON THE SELECTED PLANTING SITE?

YES

YES — IS IT A RECENTLY CREATED SITE? — NO

NO

2) IS PLANTING TO BE DONE AMONG EXISTING NATURALLY PATCHY SEAGRASS?

YES — REJECT

NO

IS THE PLANTING SITE < 0.2 ha? — NO — ARE SEAGRASSES ADJACENT TO THE SITE AT THE SAME DEPTH

YES

PLANT SITE AT DEPTHS (1) DETERMINED BY CLOSEST SEAGRASS BED — YES   NO

NO

MONITOR PLANTINGS

SEE MONITORING GUIDELINES AND REGIONAL MODULE

OPTIONS
1) CONDUCT PILOT PLANTING (2)
2) CONDUCT FULL SITE EVALUATION

CAN THE AGENT THAT CAUSED SEAGRASS LOSS ON THE PROPOSED PLANTING SITE BE IDENTIFIED? — YES

NO

OPTIONS
1) VERY HIGH RISK W/ NO MONITORING

2) EXTENSIVE MONITORING TO DET. SUITABILITY

3)   REJECT

DETERMINE SOURCE OF IMPACT FROM CATEGORY(S) BELOW:

LIGHT

NUTRIENTS (eutrophication)

TEMP. / SALINITY

MECHANICAL PERTURBATION

REJECT — NO — HAS THE AGENT OF LOSS ABATED?

UNKNOWN   YES

PLANT SEAGRASS

MONITOR PLANTINGS: SEE MONITORING GUIDELINES

Figure 2.1. Decision flow diagram for seagrass planting with an emphasis on a mitigation scenario.

Early coordination with permitting and commenting agencies is critical. Because there are typically several agencies with overlapping jurisdictions, several permits may all be required for a single planting (e.g., municipal, county, aquatic preserves, state parks, State and federal agencies). This process should certainly be started months, and preferably a year in advance of the time planting is anticipated. Because many states prohibit harvest and/or planting without permits, failure to comply with permitting requirements can result in civil penalties.

Several coordination protocols have been developed. One was designed specifically for siting marinas (Lockwood 1990) while another was designed to assist agencies collect resource information prior to commenting on a permit request (E. Nelson, NMFS, Northeast Region, pers. com.). In Nelson's protocol, two tiers of information are requested. Tier one relies on the discovery of extant information regarding the distribution, quality (e.g., biomass, density), and function of the beds (e.g., fishery surveys) in question and directs the applicant to a host of potential information sources. Should tier one efforts not provide enough resource information to proceed with processing a permit request, then a new, relatively detailed on-site assessment is triggered (tier two). The idea of a standardized protocol is essential to accurately convey the scope of the potential resource injury to the public stewards and to simultaneously treat applicants in a consistent and fair manner.

There are a number of other policies and/or activities that have been developed to aid in seagrass protection and management. Hershman and Lind (1994) have summarized the variety of programs in the Pacific Northwest which exist and placed them into six categories:

1. project review occurring at all levels of government;

2. water quality policies many of which can impact directly on seagrasses;

3. public land management policies of state, federal and tribal agencies owning submerged lands;

4. restoration/habitat development policies implemented by all levels of government;

5. damage reduction policies; and

6. inventory and mapping programs which should include, but most frequently do not include seagrasses.

They note that there is little coordination among these processes and that, in fact, many lack the necessary geographic scope to effectively protect seagrasses. A major finding is that, although federal and state jurisdiction exists over seagrasses, no specific policies for managing seagrasses exist in the Northwest. Although policies exist for other portions of the coastal zone of the United States, they often suffer from lack of coordination both among and within agencies, as noted by Hershman and Lind (1994) for the Northwest.

Such coordination protocols would be better employed if some broader, state-level policy regarding seagrasses were in place. To our knowledge there are a limited number of published policies that specifically mention or are designed to address the protection and/or mitigation of damage to seagrass habitats (Stephan et al. 1997). Here we review the Southern California eelgrass mitigation policy, EPA's Chesapeake Bay Program, and the State of Connecticut's Coastal Zone Management Program.

Southern California

The Southern California eelgrass mitigation policy was adopted in July 1991 after having been developed by federal and state resource agencies (National Marine Fisheries Service, U. S. Fish and Wildlife Service, and the California Department of Fish and Game). This policy recognizes the ecological value of seagrasses, specifically eelgrass, and lays out procedures to be used for on-site mitigation performed to compensate for adverse impacts caused by projects addressed in Section 404 permits. The recommendations for site selection, transplanting techniques, and monitoring measures are largely based on published articles of the authors of this study. The Southern California policy also recommends criteria for success which are limited to the plant component and not to the system as a whole, a point supported by Fonseca et al. (1996b).

Chesapeake Bay

The EPA Chesapeake Bay Program has developed an awareness of the value of seagrasses in the ecology of the Bay, and in July 1989 developed an Agreement Commitment Report signed by the states of Virginia, Maryland, and Pennsylvania, the District of Columbia and the Environmental Protection Agency entitled "Submerged Aquatic Vegetation Policy for the Chesapeake Bay and Tidal Tributaries." This agreement states that the signers will work together to implement four major areas relative to seagrasses. These include assessment of the distribution and abundance of the resource, development of protection and restoration guidelines, and implement an education component to increase public awareness of the value of the resource. The educational component recognizes the need for scientific research to

improve our knowledge and understanding of submerged aquatic vegetation to ensure that efforts to protect and restore this resource continue to be effective. It is refreshing to see that recognized in a ecosystem or watershed management approach such as is being conducted in the Chesapeake Bay.

The Submerged Aquatic Vegetation Workgroup of the Chesapeake Bay Program developed a guidance document for protecting submerged aquatic vegetation from physical disruption (Chesapeake Executive Committee 1995). As part of this guidelines document, the workgroup has summarized policies and activities of the states of Maryland and Virginia, the District of Columbia, and four federal agencies that directly impact the health of submerged aquatic vegetation in the Chesapeake Bay (U. S. Army Corps of Engineers, U. S. Environmental Protection Agency; U. S. Fish and Wildlife Service, and the National Marine Fisheries Service) principally as they pertain to permit applications under the Clean Water and Rivers and Harbors Acts. The reader is referred to this document for specific actions taken by these states and federal agencies.

Connecticut

The General Statutes of Connecticut, for Sections 22a-90 through 22a-112 for the Connecticut Coastal Management Act (revised January 1, 1993), presents legislative goals and policies which include insuring that the development, preservation or use of the land and water resources proceeds in a manner consistent with the ability of these resources to support development, preservation, or use without significantly disrupting either the natural environment or sound economic growth. This Act also recognizes the need to conduct and sponsor research to improve the information base upon which these decisions are made. The Act states that policies include managing estuarine embayments to "protect, enhance and allow natural restoration of eelgrass flats except in special limited cases; notably shellfish management..." While this is the only direct statement related to seagrasses, the document states that adverse impacts include "...degrading or destroying essential wildlife, finfish or shellfish habitat through significant alteration of the composition, migration patterns, distribution, breeding or other population characteristics of the natural species or significant alteration of the natural components of the habitat..."

Although many other states have developed policies relating to seagrasses, the few reviewed here differ from the other to some extent. However, they are all consistent in that they place an unquestionably high value on the maintenance of seagrass (or SAV) ecosystems, a position that signals the intent of the resource agencies to deal seriously with the resource and that allows potential developers a better understanding of the serious nature of an injury to these ecosystems.

ASSESSMENT OF INTERIM LOSSES

Another goal of many seagrass plantings is an attempt to recoup *interim loss of ecosystem functions.* This was mentioned earlier as an attribute of functional equivalency. Because the concept of success and functional equivalency are so closely tied, planning for successful restoration and/or mitigation requires early incorporation of interim loss considerations. The manner in which interim loss has been addressed historically has been through adjusting replacement ratios (how much acreage to plant per unit acreage lost). However, the manner in which interim ecosystem losses have been computed has not been consistent. Replacement ratios of less than 1:1 to as high as 5:1 have been proposed (Fonseca et al. in press), based on a number of criteria, but that ratio is usually inversely proportional to the degree which a project was in the public interest.

To compute losses though, requires some assessment of not only acreage lost but also of how long a time the functions of that acreage were lost to the ecosystem at large before it was returned to pre- or un-impacted levels. Depending on how long one wishes to amortize a loss will influence how much replanting must be done. In theory, if one hectare of seagrass were destroyed today and three hectares were replanted tomorrow and reached standards of equivalency in three years, then after those three years the planting would have largely compensated for the total loss of production; the net loss of production over this three year period would be very low. However, things rarely work this way. First, it is very difficult to consistently locate and successfully create new seagrass habitat that meets our site selection criteria (which precludes simply substituting naturally unvegetated bottom for vegetated bottom). Finding large acreage for planting in close proximity to the impacted area is rare; this means that planting is often done at a site physically removed from the impact area and any functions affected by spatial elements of ecosystem linkages (i.e., geographic setting) are lost. Second, the production that was lost was removed from a specific point in time; ecosystem functions were disrupted and those specific resources are not replaced, such as that year's spawn of herring (e.g. as in the Pacific Northwest). Further, if there was a greater hiatus between the time of impact and recovery, then one could argue that plantings conducted longer after an impact or further away from an impact have less value than ones conducted sooner or nearer. This realization is the basis for new approaches by NOAA to quantitatively standardize the interim loss problem (Fonseca et al. in press).

The assessment strategy to calculate interim loss is based on four steps of analysis:

1. documentation and quantification of the injury,

2. identification and evaluation of restoration options,

3. scaling of the restoration project to compensate for the injury over time, and

4. determine the appropriate means of compensation (e.g., monetary or planting).

The scaling aspect is the portion of the process that helps standardize the way in which interim losses are computed, irrespective of the habitat type involved. Interim lost services can be considered to be the integral of service lost from some baseline level over time (Figure 2.2). To compare services lost with those recovered by some remedial action (such as planting seagrass), the product:

square m of habitat lost x time = square m—years

is set against square m—years of services provided by the planting project, but discounted as a function of time since the initial injury (Figure 2.3). Discounting is a accepted economic principle, used to transform monetary or service flows over time into present value terms for purposes of comparison. Plantings that occur longer after an impact are discounted more than plantings conducted shortly after an impact and therefore more planting must be done as more time elapses. The NOAA Damage Assessment and Restoration Program is currently applying this procedure to seagrasses and it now has been upheld in court (U.S. District Court, 92-10027-CIV-DAVIS). Initial results appear promising but require an empirical assessment of rates of recovery by seagrass. These rates are poorly known and experiments have been implemented to provide additional data. Population growth and coverage rate information has been useful (*sensu* Fonseca et al. 1987a,c) and supports previous claims for the need to collect these kinds of data as part of any monitoring of seagrass plantings (Fonseca 1989a, 1992, 1994)

PRE-IMPACT AND PRE-PLANTING SURVEYS: IDENTIFYING PRESENCE, ABSENCE AND REASONS FOR ABSENCE OF SEAGRASS COVERAGE

Lockwood (1991) provided guidelines for surveying sites prior to impact and how to interpret these data to plan subsequent plantings. Essentially, any quantitative survey method will work, such as line transects or grid sampling, but some basic quantitative standards such as presence/absence of a seagrass species over known areas must be met. Sampling for the presence/absence of seagrass should encompass the entire impact site on the closest spacing practicable and clearly specify the range over
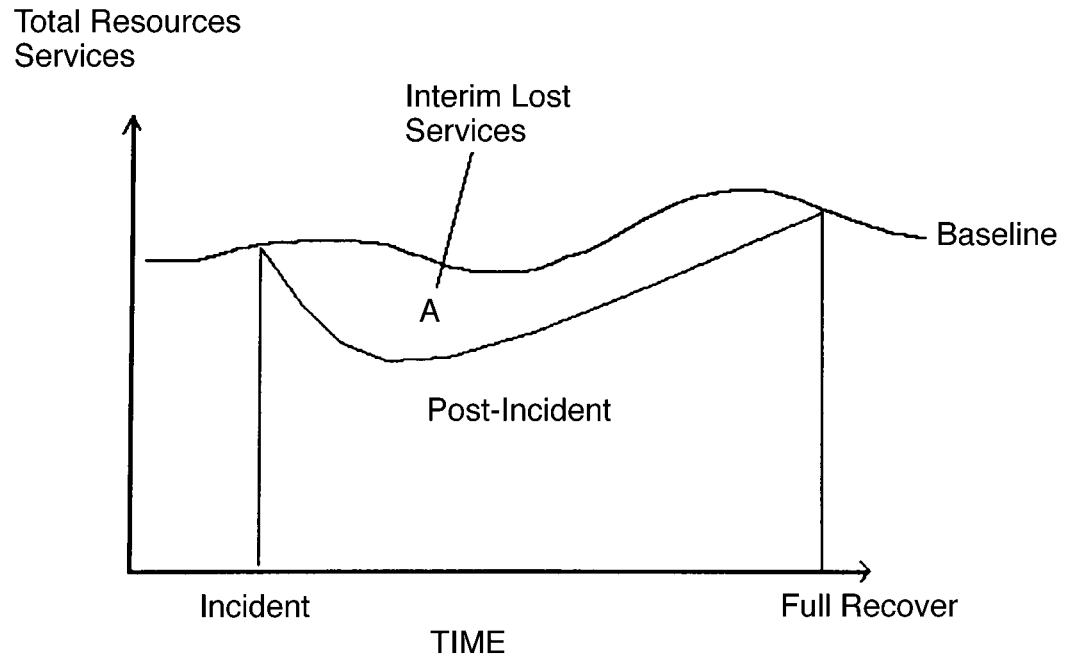
Figure 2.2. Diagrammatic representation of the computational process used for assessing interim loss of seagrass habitat functions (from B. Julius, DARP/NOAA).
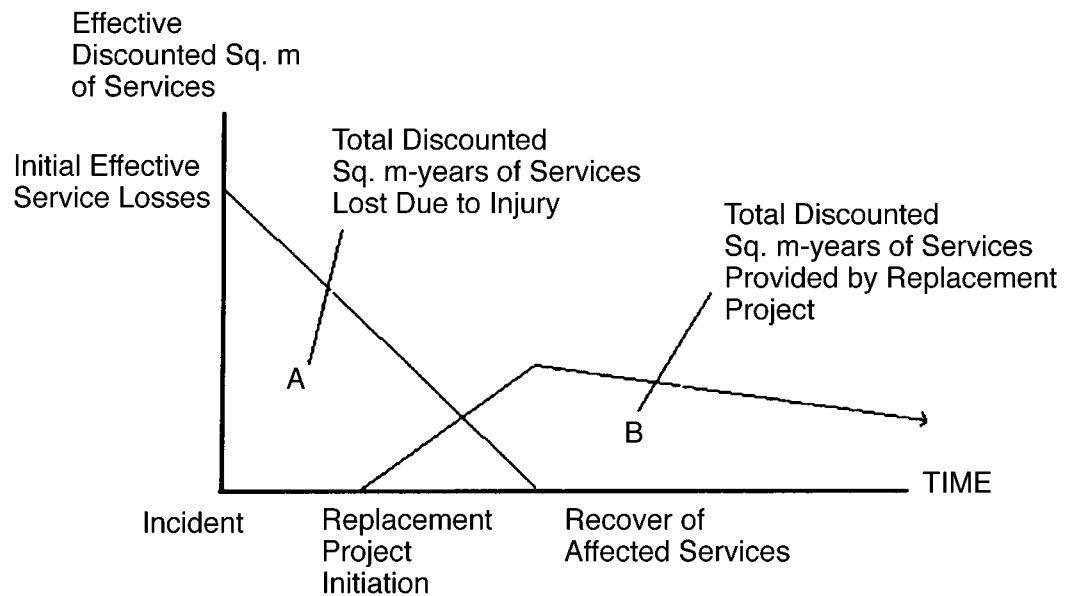

Figure 2.3. Diagrammatic representation of the discounting process used to compute replacement ratio for planting seagrass under a mitigation scenario (from B. Julius, DARP/NOAA).

which sampling is to be done and the resolution with which samples are to be taken. For example, the following description would be considered adequate for such a report:

On (dates) [representing spring and summer growing conditions], five 100-m transects spaced 20 meters apart were swum by divers over the site who then recorded the average percent cover seagrass [percent of 16, 25 x 25 cm subunits within a 1 m² quadrat that contained a minimum of 1 seagrass shoot] where 1 m² quadrats were assessed on 10-m intervals along each transect). Alternatively, a video camera may be towed over a transect line marked at 1-m increments and the cover estimated using a point-grid method (e.g., Braun–Blanquet 1965, Virnstein 1995).

The depth distribution and coverage of each species present should be recorded. If seagrass occurs as small isolated patches of grass among open, unvegetated areas, then the sum total area occupied by the patches (portion of the sea floor where rhizomes overlap) could be recorded per unit seafloor (where the unit area of seafloor is at least a minimum of 100 m²). If plants are very large and separated from each other then the aforementioned quadrat method will be more appropriate for assessing coverage. Data on species composition should be used to guide the selection of species for later planting. Further, these data can be used to determine the amount of seagrass that can be salvaged for planting other sites or potentially stored for replanting onto the original site if the disturbance is short-lived.

Aerial photographs of appropriate resolution can provide useful information for evaluating existing seagrass beds. A time series of aerial photographs, (preferably ~1:20,000 scale) if available, can be particularly useful in determining the dynamic nature of a site. Photographs should be used only if taken during the peak biomass season for the seagrass in question. Moreover, it is sometimes difficult to accurately determine the lower depth limit of seagrass on a site from aerial photographs. Lower depth limits of seagrass distribution should be verified by on-site inspection, especially if bottom features which are clearly deeper than the apparent lower limit of the seagrass in the picture cannot be discerned in the photographs. However:

If aerial photographs taken over a ten-year period indicate no history of seagrass cover, then the potential planting site should be regarded as marginal, or better, rejected.

Episodic seagrass cover on a potential planting site, either among years or seasons (as might be the case with seed recruitment) would suggest that planting there would only pulse the system and not provide sustained habitat replacement. As these caveats

imply, unlike many other wetlands where site engineering is often an option, it is very difficult to locate a planting site that will provide self-sustaining seagrass habitat. Also, as discussed under the section titled "Spatial Scale and its Role in Defining Seagrass Habitat," aerial photography must be taken with sufficient resolution to detect the smallest patches of seagrass in the target area; this may require fairly low overflights (< 1000 feet) which may present significant problems in certain areas where Federal Aviation Administration rules prohibit low flights. S. Nixon and B. Kopp (Graduate School of Oceanogr., Univ. Rhode Island, Narragansett, RI., pers. com.) have employed Geographic Information Service technology to overlay water column light transmissivity, water depth, wave exposure and other factors in a site selection process that may become a model approach for regional restoration planning.

MINIMUM SIZE TO JUSTIFY PRE-PLANTING MONITORING

Environmental data or pilot test planting results should be collected to provide an indication of planting success prior to the commitment of the entire project's resources. However, personal observations suggest that some plantings may be sufficiently small (~500-1000 planting units) so the cost of collecting environmental data and performing pilot plantings are equivalent to the cost of planting the entire site itself.

PLANTING SITE SELECTION AND OFF–SITE VS. ON–SITE PLANTING

Selecting an appropriate planting site is perhaps the single most important step in the entire process. It is also the step that is the most difficult to objectively verify. This is because the circumstances contributing to the presence or absence of seagrass at a given site vary tremendously (see "Pre-Impact and Pre-Planting Surveys," above and criteria in Appendix E, p. 211). Planting areas may be classified as either on- or off-site. When an off-site planting area must be selected, whether it be for restoration or mitigation, it must pass a simple, but exacting, test: "If seagrass does not currently exist at the (chosen) site, what makes you believe it can be successfully established?" (Fredette et al. 1985).

The absence of seagrass on what may appear to be an otherwise suitable site often indicates some inherent difficulty in colonization or a temporally dynamic site (e.g., as the result of disease, F. Short, Jackson Estuarine Lab., Durham, NH, pers. com.). In the case of disease-induced loss of cover, planting may be considered sim-

ilar to planting among existing seagrass patches in that temporarily freed–up bottom space would be used as an inappropriate planting site. Planting among patches of existing natural seagrass should also be rejected because this too will only pulse the system and not create any long–term increase in seagrass acreage and this space is soon required by the spatially dynamic seagrass patches (see sections on "Defining Seagrass Habitat" in Chapter 1 and "Constraints Imposed by Physical Setting on Planting Operations," below). The take–home message is that if one contemplates off–site compensatory mitigation, there are usually few, if any sites available that: a) can support seagrass growth and, if they do, b) do not involve habitat substitution, or c) do not satisfy the no–net–loss rule.

One form of off–site planting that meets the criteria discussed above is grading down uplands to elevations suitable for planting. Although this entails the trade–off of upland habitat for seagrass, if that upland is zoned for development, then its conversion to seagrass habitat type is warranted. Other off–site options include filling of dredged areas or areas that have experienced an improvement in water quality (e.g., transparency, temperature, etc.). These latter two choices, however, may include areas which historically supported seagrasses and thus may not be effective to offset seagrass loss in compensatory mitigation (see section "Pitfalls in the Mitigation and Restoration Process"). In the case of on–site planting associated with a particular project (i.e., planting back into a portion of the site which suffered a loss of seagrass), the activity which originally caused the loss of seagrass must have ceased.

ENGINEERED SITES

In many instances seagrass planting takes place on sites that have met the criteria of past seagrass presence, identifiable (and human–induced) agents of loss, and the termination of those loss agents (Thayer et al. 1985). Less frequent, however, is planting on sites that have been specifically engineered to accommodate seagrass planting. Those that have been contoured to appropriate elevations have had good success; in the Laguna Madre, the interiors of dredged material islands were returned to subtidal elevations suitable for seagrass growth and connected to the adjacent sound; both plantings and natural recolonization were successful (Montagna 1993). Work in San Diego Bay in the early 1980's featured the creation of a submarine dike that allowed placement of dredge material shoreward of the dike, raising the bottom to elevations (~ –5 m) with suitable light for seagrass growth (pers. obs.). Short (1993) conducted a similar planting in Great Bay, NH. Both projects created viable seagrass habitats that are currently supporting extensive seagrass cover, even though portions of the New Hampshire site was susceptible to winter ice shear. The drawback to this approach is that naturally unvegetated subtidal seafloor was converted to seagrass habi-

tat; such habitat substitution may not be an acceptable mitigation tradeoff as implied by recent symposia on the important ecological roles performed by unvegetated estuarine sediments (Marine and Estuarine Shallow Water Science and Management in the Mid-Atlantic Region, Atlantic City, NJ).

Other engineered sites include those near Beaufort, NC, where seagrass and salt-marsh habitats were created on old dredged material islands for the purposes of both stabilizing eroding shorelines and experimentally investigating recovery trajectories and linkages among these two habitat types (Fonseca et al. in press.). In this case sites that did not receive subsequent dredged material successfully supported seagrass plantings. Thus, sites have been engineered to support seagrass mitigation and have enjoyed good planting success. Such attention to site preparation is encouraged although the costs in performing such site engineering may often place it outside the realm of possibility for small mitigation projects.

CHANNEL PLANTINGS, EFFECTS OF STRUCTURES, AND OTHER HUMAN ACTIVITIES

On-site planting often entails planting into permanently modified areas, such as in the case of channel dredging, and typically cannot accommodate a replanting ratio (planted seagrass area/lost seagrass area) above 1:1. Planting along banks of artificial-ly-created channels is logical if the depth of planting does not exceed that at which the plants occurred prior to dredging even though a larger potential planting area may be created. Channel margins, however, are highly susceptible to subsequent grounding events by vessels which will limit replanting options. In addition, many channel bottoms at navigational depths will not support seagrass due to insufficient light or severe scour from propwash. Channels, being deeper than the surrounding seafloor often act as areas of enhanced deposition, especially of organic material. As a consequence, highly reducing sediments and prolonged periods of anoxia may be found in channels which are typically highly stressful, if not lethal conditions for sea-grasses. As a general rule, planting in and around channels is very risky.

As an alternative to dredging access channels, docks are often built to access ves-sels from land. Docks, however, have their own suite of potential impacts to seagrass beds. Besides actual impacts where dock pilings are installed, unless a dock is narrow and high above the water, it will create a substantial shadow that will reduce seagrass density and biomass, as seen in Massachusetts (Burdick and Short 1995). At the deep-er end of the seagrass bed, plants will be killed as the shadow pushes them below

compensation irradiance (see section on "Light Requirements for Transplanting," below). Relationships derived by Burdick and Short (1995) in New Hampshire reveal that at those latitudes and tidal regimes, a 1-m wide dock had to be nearly 5 m high to maintain eelgrass (*Zostera marina*) bed quality. Burdick and Short (1995) also found that plank spacing and width were far less important than height of the dock and its compass alignment (north–south docks had less impact than east–west docks because their shadow moved further across the bed, thus mitigating the shading effect). Burdick and Short (1995) recommend that T-shaped docks, with a floating dock beyond the outer edge of the seagrass, minimizes collateral disturbance from propwash and boats settling onto or perpetually shading beds when they are moored at the dock. Similar findings were reported by Loflin (1995) in Florida. While existence of collateral disturbance (e.g., dispersing of fish and wildlife) are not well-documented in seagrass systems, there is also no reason to expect that such disturbances would not occur. These kinds of empirical estimates are greatly needed by resource managers nationwide but must be developed on a regional basis.

Other collateral direct impacts to seagrass beds caused by human activity include propeller scarring and mooring chain scars. Sargent et al. (1995) have found thousands of acres of seagrass beds in Florida either destroyed or significantly degraded as the result of vessel scarring, which includes not only vessels with propellers but jet skis as well (pers. obs.). The source of injuries to seagrass beds is varied, but collateral impacts from otherwise seemingly benign activities such as foot traffic or mooring a vessel to those actually designed to minimize or avoid impacts to seagrass beds (docks) can sometimes result in significant damage. Care must be taken to avoid these impacts.

CONSTRAINTS IMPOSED BY PHYSICAL SETTING ON PLANTING OPERATIONS

If significant physical alteration such as dredging has occurred at a site, on-site plantings often cannot provide sufficient acreage to prevent a net loss of habitat. Another common, but less obvious physical alteration occurs when bulkheads are installed. Many bulkheads are designed as walls to efficiently reflect waves. This wave reflection effectively doubles the wave energy seaward of the wall, often eroding existing offshore beds and creating a situation where they likely cannot be replanted. When physical alterations are subsequently ameliorated, on-site planting is appropriate and offers one of the few circumstances where substantial acreage can be generated, even though historical levels may not be attainable.

The physical setting will dictate the amount of seagrass coverage per unit sea-floor as well as the pattern of that coverage. The organization of the coverage into patches is correlated with wave and current climate (Fonseca et al. 1983). Application of wave and current data into site layout is given below in the section, "Spacing of Planting Units." Physical setting is correlated with sediment stability which can severely limit planting success (Fonseca et al. 1985). In general, sites with high wave exposure and tidal current speeds are difficult to restore because of frequent disturbance.

A precise survey of the physical conditions at the planting site will assist in determining the amount of plant material required later. In the case of mitigation projects, a similarly precise survey of conditions prior to any proposed impact is required to obtain an accurate estimate of the seagrass habitat to be lost. This will allow accurate computation of planting ratios (mitigated acreage vs. impacted acreage) to be computed. For example, if 0.5 acres of continuous cover seagrass bed (i.e., low-energy site) were lost to a project and a high-energy planting site was chosen which would typically support patchy beds one would have to:

1. anticipate planting an acre of bottom to achieve 0.5 acres of cover, and

2. budget for substantial replanting (as much as 50 percent of the original planting) because planting failures increase with higher currents (Fonseca et al. 1985).

We focus on wave and tidal current effects on seagrasses in unconsolidated sediments because we have no quantitative information regarding seagrass growing on consolidated sediments (e.g., *Phyllospadix* spp.) to guide selection of a physical setting. We draw the reader's attention though to work by Turner and Lucas (1985) who outlined spatial and temporal dynamics of a rocky intertidal seagrass community and work by Sousa (1979) who noted an inverse relationship between boulder size and frequency of disturbance, an important factor to recognize if plantings are attached to boulders.

For seagrass growing on unconsolidated sediments, we have some data from southern Core Sound, North Carolina, and Tampa Bay, Florida, to indicate a strong relationship between wave exposure (described below) and tidal current speed. To obtain these relationships in North Carolina, seagrass coverage data was determined by mapping 18, subjectively chosen seagrass study sites in Core and Back Sounds, Carteret Co. (latitude 34.40-34.50 N, longitude 76.20-76.40 W). These sites were chosen based on examination of aerial photographs and ground-truthing to repre-

sent the full range of seagrass coverage that could be locally identified. These sites are also colonized by a mixture of *Zostera marina* and *Halodule wrightii*. Carteret Co. represents the primary area of overlap of these two seagrasses on the east coast of the United States. The occurrence here is marked by different seasonal peaks of abundance (Thayer et al. 1984).

Exposure to waves was calculated for each site using methods of the Shore Protection Manual (1977) and Keddy (1982) as reported by Murphey and Fonseca (1995):

$$\text{Exposure} = \sum_{i=1}^{8} (V_i \times P_i \times F_i)$$

where:
    i = i^{th} compass heading (1–8)
    V = average monthly maximum wind speed in m s^{-1}
    P = percent frequency which wind occurred from the ith direction
    F = effective fetch

To analyze the effect of both forms of water motion (waves and currents) in North Carolina, exposure values (based on mean monthly maximum wind speeds), and tidal current speed (peak free-stream speed over seagrass at the lunar maxima) were plotted against percent coverage using all the sites from 4 sampling times covering 2 years. The percent coverage for each site was calculated by dividing the total number of one meter square (pixel) observations that had seagrass by the total area of the survey plot (2500 m^2). Site maps were produced from the survey data and each pixel registered as containing seagrass plotted as a square.

These surveys produced significant relationships as seen in Figure 2.4 (Fonseca and Bell in press):

Percent Cover = Exposure index (-0.0135) + 92.525
r^2 = 0.45

Percent Cover = Maximum monthly tidal current speed in
cm s^{-1} (-2.644) + 111.044
r^2 = 0.60

We are not sure how these data may be applied to other areas because to our knowledge, seagrass plantings have not been conducted to account for anticipated
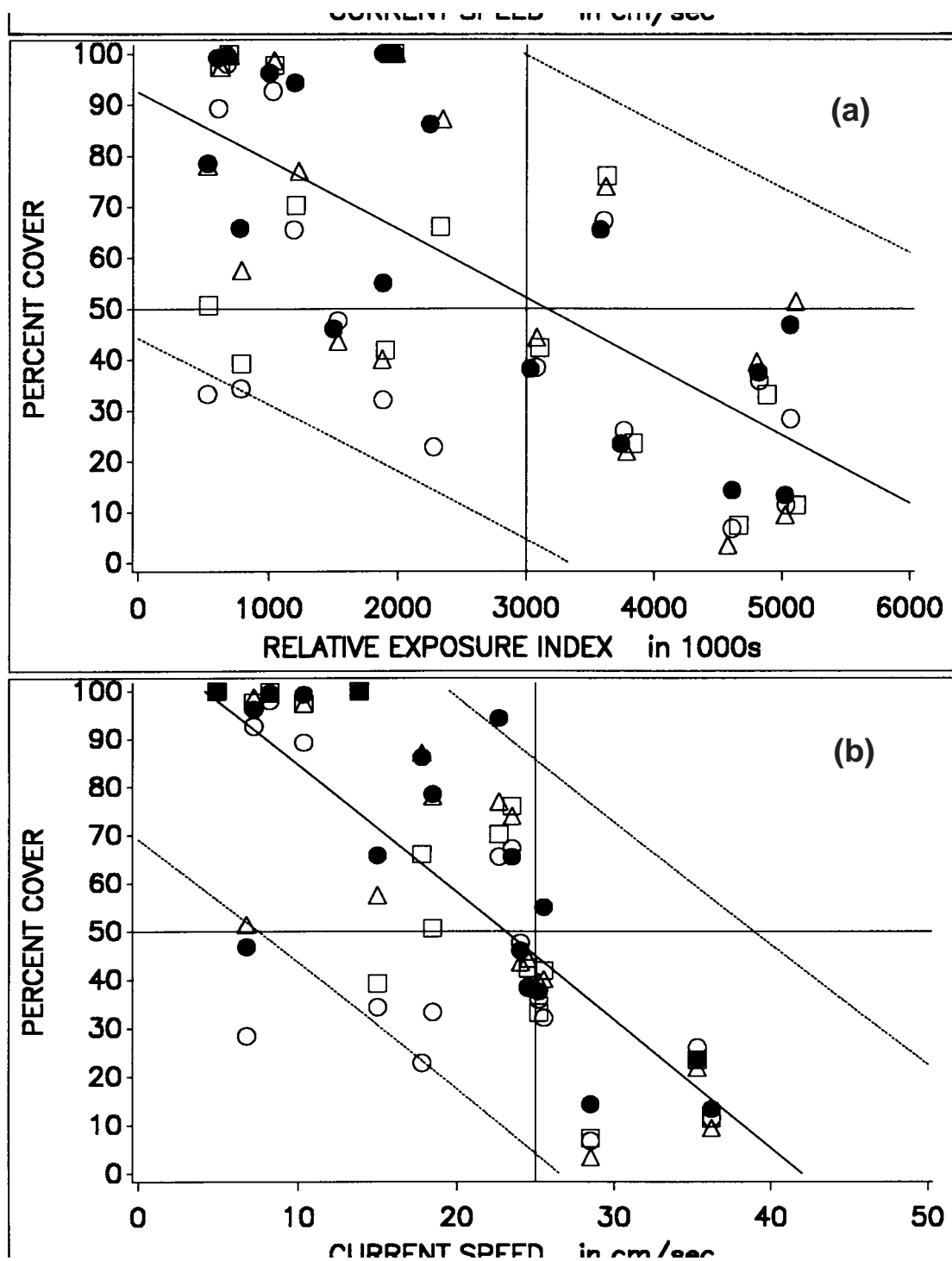
Figure 2.4.   Relationship between seagrass cover and physical setting for seagrass beds near Beaufort, NC. (a) Relationship between seagrass cover and exposure index (eq. 1 in text) from eighteen, 50 x 50m plots surveyed with 1m resolution. (b) Relationship between seagrass cover and tidal current speed from eighteen, 50 x 50m plots surveyed with 1m resolution.

76

landscape patterns. We suggest that in areas where currents speeds are less than 15 cm s^{-1}, only wave exposure be considered in predicting coverage. For lack of better guidance, but taking into account observations of dune formation even in subtropical areas (Fonseca 1996a), we suggest that the tidal current speed model hold for seagrasses in all settings when currents are greater than 15 and less than 50 cm sec^{-1} (tidal current speed > 50 cm sec^{-1} would indicate rejection of the site, Figure 2.5). It should be noted that application of these data are extremely experimental but may represent an important aspect of planning that might be considered in planting operations.

EMERSION EFFECTS

Despite the long-standing recognition of seagrass emersion as a factor in its ecology (Johnson and York 1915, cited in Harrison 1982), until recently little work has been done to document direct effect of emersion on the plants. The effects of emersion vary widely around the U.S.; in the southeast and Gulf states, emersion can cause significant mortality. In northern states eelgrass beds may be regularly exposed at low tide but avoid serious desiccation due to local micrometerological factors, such as fog, cool air temperatures, and high local humidity in the immediate vicinity of the seagrass canopy. Moreover, seagrass beds can also trap water in their canopy as the blades lay over at low tide by making the path for water drainage extremely long by causing flow to wend through the leaves and stems. Powell and Schaffner (1991) reported this phenomena in Florida Bay and it has also been observed in New Hampshire (F. Short, pers. com.) and elsewhere (authors' pers. obs.). In addition, Bulthuis et al. (1984) demonstrated that seagrass beds retain both fine sediments and nutrients while trapping water. Limited work by Harrison (1982) on comparative emersion effects among *Zostera* spp., and more detailed studies by Cooper and McRoy (1988), Cooper (1989) on isotopic variation with emersion, and Perez-Llorens and Niell (1993) on *Zostera* spp. may constitute the entire body of quantitative work on the subject. Perez-Llorens and Niell (1993) describes perhaps the only work on non-rocky intertidal seagrass where an experiment was designed specifically for the effects of temperature and emersion. In that study, photosynthetic rates were significantly higher in water than in air, but a narrow-leaved morphotype of *Zostera noltii* displayed higher photosynthetic rates than a broader-leaved morph. Increased temperature decreased photosynthesis during the 2-h incubation period for both morphs, although the narrow-leaved morph was more resistant. However, emersion time was not varied and recovery of photosynthetic capacity was not measured. Adams and Bate (1994) removed individual blades for desiccation effects and measured chlorophyll fluorescence. They found that the wider-bladed *Zostera* was significantly more
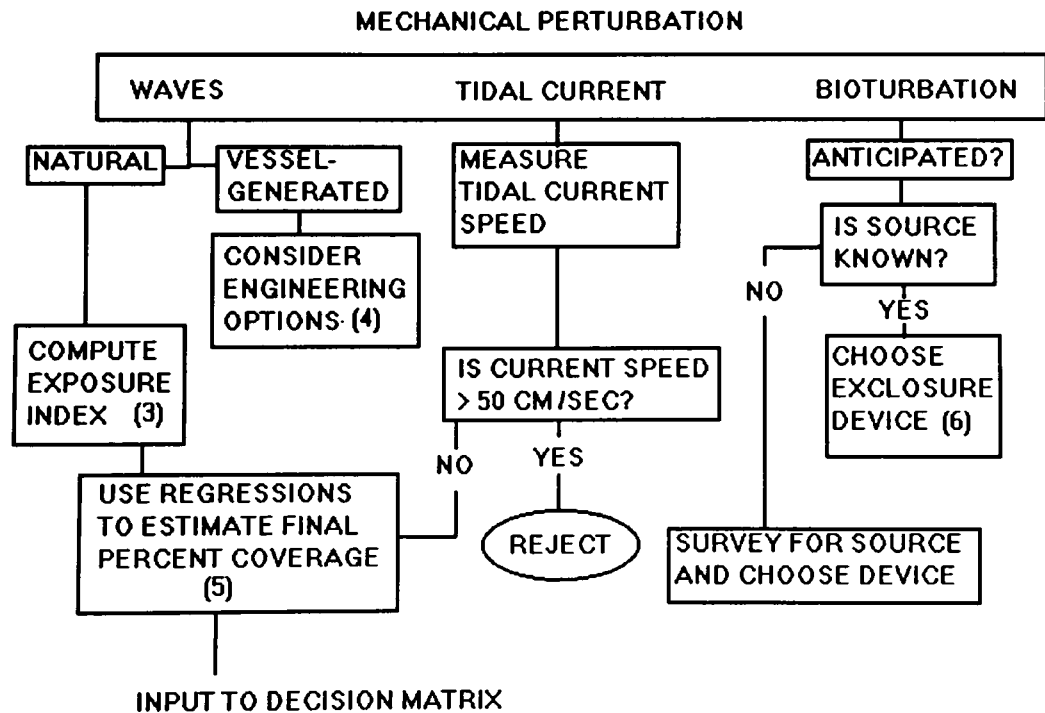
MECHANICAL PERTURBATION

```
┌──────────────────────────────────────────────────────────────┐
│   WAVES              TIDAL CURRENT            BIOTURBATION     │
└──────────────────────────────────────────────────────────────┘

┌─────────┐ ┌──────────┐  ┌──────────────┐      ┌─────────────┐
│ NATURAL │ │ VESSEL-  │  │ MEASURE      │      │ ANTICIPATED?│
└─────────┘ │ GENERATED│  │ TIDAL CURRENT│      └─────────────┘
            └──────────┘  │ SPEED        │         ┌───────────┐
         ┌──────────────┐ └──────────────┘    NO   │ IS SOURCE │
         │ CONSIDER     │                          │ KNOWN?    │
         │ ENGINEERING  │                          └───────────┘
         │ OPTIONS (4)  │                             YES
         └──────────────┘ ┌──────────────┐      ┌─────────────┐
┌─────────┐               │ IS CURRENT   │      │ CHOOSE      │
│ COMPUTE │               │ SPEED        │      │ EXCLOSURE   │
│ EXPOSURE│               │ > 50 CM/SEC? │      │ DEVICE (6)  │
│ INDEX(3)│               └──────────────┘      └─────────────┘
└─────────┘                 NO    YES
   ┌──────────────────┐                      ┌──────────────────┐
   │ USE REGRESSIONS  │      ( REJECT )       │ SURVEY FOR SOURCE│
   │ TO ESTIMATE FINAL│                       │ AND CHOOSE DEVICE│
   │ PERCENT COVERAGE │                       └──────────────────┘
   │ (5)              │
   └──────────────────┘
```

INPUT TO DECISION MATRIX

Figure 2.5. Site selection process using physical setting.

resistant to desiccation than narrower blades of *Ruppia,* although tests were conducted on blades in isolation and thus did not account for the possible mitigative effects of a canopy or moisture content of the sediment on desiccation.

The findings of Perez-Llorens and Niell (1993) have implications in other regions. For example, in the Beaufort, NC area, two seagrass species occur at the edge of their distributions — the temperate species, *Z. marina* and the subtropical species, *H. wrightii. Zostera marina* leaves are approximately 3-5 mm wide whereas *H. wrightii* leaves are 1-2 mm wide. The narrower-leaved species can exist in slightly shallower water which receives more frequent emersion (*sensu* Perez-Llorens and Niell 1993). Thus, the interaction of species and canopy morphology with tidal regime may be a significant zonation factor as has been documented for macroalgae. If this is the case, then the ability of the narrow-leaved form of *Zostera noltii* to maintain higher photosynthetic capacity at higher temperatures might result from an adaptive advantage for narrow leaves in regulating leaf temperature. These findings are contradicted by those of Adams and Bate (1994) where narrow-leaved species were less resistant to desiccation. Clearly experimental designs that simulate natural settings are needed to generate quantitative measures of desiccation tolerance of sea-

grasses. Knowledge of desiccation tolerance will facilitate selection of appropriate seagrass species for a site in an ecoregion where such choices may be possible.

Despite contradictory experimental findings, exposure to air is often detrimental to most seagrasses. Human alteration can render many sites too shallow for seagrasses. For example, reduced water depth from dredge material deposition may result in sites where transplants would be exposed at low tides, causing them to desiccate. Sufficient water depth must be maintained to cover the plants even at lowest tides. Very short term events (2-3 hours) may substantially alter seagrass abundance and distribution. For example, desiccation caused by an extreme low tide at mid-day in the summer can determine the upper limit of seagrass distribution in an entire bay for the following year (Beaufort, NC, pers. obs). Areas with high turbidity and tidal amplitudes are extremely difficult to plant given that desiccation at low tide and light extinction at the lower depth limit must both be avoided (E. Koch, Horn Point, MD, pers. com., *sensu* Koch and Beer 1996). Because plantings are generally more stressed than established beds, we suggest (as we will for light) that emersion should be avoided. Arranging planting depth for minimum emersion is best planned by surveying elevations of nearby beds.

Dennison and Kirkman (1996) suggest a balance of tidal elevations for seagrass survival based on the premise that Secchi depth is equal to the compensation point for some seagrasses, while considering tidal range and type. For seagrasses existing intertidally (with the possible exception of *Phyllospadix* spp.) mean range of astronomical tides (MTRA) must be greater than the mean range of barometrically-driven tides (MTRA > MTRB) for their survival. Also, Secchi depth (Zd) must be greater than MTRA (Zd > MTRA). For subtidal seagrasses, Zd > MTRA and Zd > maximum depth of seagrass distribution (Z; Zd > Z) for seagrass survival. This model has not, to our knowledge, been tested in North America.

BIOTURBATION

Prior to rooting and coalescence of plantings, seagrasses are especially vulnerable to bioturbation (Fonseca et al. 1994). Bioturbation is widespread and has been shown to limit distribution of natural beds as well. Ogden et al. (1973) documented the effect of the echinoid *Diadema antillarum* on the formation of grazing halos in seagrass beds associated with West Indian patch reefs. Similarly, Camp et al. (1973) and Valentine and Heck (1991) demonstrated the role *Lytechinus variegatus* in producing unvegetated areas from seagrass beds in the northern Gulf of Mexico. Orth (1975) attributed the destruction of large areas of eelgrass (*Zostera marina*) in the

Chesapeake Bay to the feeding activity of the cownose ray (*Rhinoptera bonasus*). Suchanek (1983) and Harrison (1987) demonstrated the negative impacts of the burrowing shrimp *Callianassa* on the seagrasses *Thalassia testudinum* and *Zostera* spp., respectively. Valentine et al. (1994), however, found that even large animals such as rays were apparently unable to create unvegetated patches within existing *Thalassia testudinum* beds, and that only very large rays were capable of producing pits at the bed-sand margin that resulted in damage to these seagrasses' rhizomes. They also found that sand dollars (*Mellita quiquiesperforata*) did not disturb these edges whereas stone crab (*Menippe* spp.) burrows were disruptive. They point out that the deep rhizome layer of *T. testudinum* (as compared to *Z. marina* or *H. wrightii* as found in the Beaufort, NC area) may insulate these plants from ray and sand dollar disturbance. Various waterfowl can graze down seagrasses (e.g., Black Brant, redhead ducks, mallards, etc.) (Thayer et al. 1984) and can destroy early stage plantings (Beaufort, NC, pers. obs.). If waterfowl grazing is anticipated, then exclosures should be covered on top and not just on the sides. Bioturbation has been linked to maintenance of fragmented seagrass landscapes (Townsend and Fonseca 1998).

Bioturbation is a factor that can require a substantial replanting budget; thus some kind of exclusion device is often needed. For example, Fonseca et al. (1994) found that in areas of Tampa Bay where currents did not exceed 13 cm/sec, a greater than 50 percent loss of planting units occurred due to sediment disturbance, apparently by rays. Fonseca et al. (1994) found that caging of *Halodule* plantings with one inch mesh galvanized chicken wire cages (sides and tops) in Tampa Bay made a difference of < 1 percent survival with no cages versus 60 percent survival with cages. Merkel (1988a) also found extensive disturbance of seagrass transplants in San Diego Bay and used stakes, fencing, and erosion matting in an attempt to improve planting survival. Short (1993) constructed gill-net cages with no tops which excluded horseshoe crabs and green crabs and preserved eelgrass plantings in a New Hampshire estuary. However, Short (pers. comm.) has also reported that certain polychaete worms (e.g., *Nereis*) will pull the blades of early stage eelgrass plantings down into their burrows to feed on epiphytes. This lays the short shoot along the sediment surface where it is then subject to other attacks and burial. Short found that decreasing planting density from 0.5 m on center to 0.1 m centers resulted in slower, but still complete incorporation of blades into burrows. We are not aware of a remedy for this source of bioturbation.

Bioturbation events can occur quickly. We have experienced 100 percent loss of *Halodule* and *Syringodium* planting units within 24 hours of planting (Florida Keys backreef area) due to grazing where chicken wire cages were not used. The lack of good pre-project information on bioturbation potential will usually cost one more

in remedial planting than will be saved from planting a minimum of material for a test of bioturbation at the onset.

SEDIMENT THICKNESS

Insufficient sediment thickness (e.g., bedrock too near the surface) has been shown to be limiting to the distribution of some seagrasses (Zieman 1982b), particularly *Thalassia testudinum*. Although not documented for any other seagrass, the potential for exposure of bedrock by currents due to shallow unconsolidated sediments should be considered when choosing a site. In relatively quiescent areas, we have successfully established *H. wrightii* and *S. filiforme* beds on sites with as little as 15 cm of loose carbonate sand over bedrock (Fonseca et al. 1987a). Generally, species with shallow root and rhizome systems (e.g., *Halophila*, *Halodule*, *Zostera*) may not be inhibited by thin veneers of sediment.

SEDIMENT STABILITY: EROSION AND BURIAL OF SEAGRASS SHOOTS

Generally, sediment stability is going to be correlated with wave exposure and tidal current speed. The relation between exposure and/or currents and sediment stability is difficult to predict given variation in sediment grain size (therefore different erosion thresholds) among sites and the episodic nature of wind events. The apparent threshold responses evident in Figure 2.4 suggest, however, that some erosion threshold may be represented by exposure indices near 3×10^6 and tidal current speeds of 25 cm sec^{-1}. Merkel (1992) suggests that erosion rates of 0.5 mm day^{-1} and burial rates of 0.3 mm day^{-1} are limits for *Z. marina* survival on the West Coast. These data compare favorably with the sediment fluctuation limit of ~1.0 mm day^{-1} found on the East Coast for both *Z. marina* and *H. wrightii* (Fonseca et al. 1985).

Conversely, there are few data to indicate critical burial depths; those depths likely vary among species. However, our preliminary data for *H. wrightii* (unpubl. data) indicate that when 25 percent of the shoot is buried, 75 percent of the plants survived, but when 75 percent of the shoot was buried only 5 percent survived. This response suggests an exponential decline of survival with percent burial. We are aware of no data on North American species that gives guidance on the duration of burial time after which recovery of the plants would be expected (but see Marba and Duarte 1995, Terrados et al. 1998).

Prior to planting, measurement of sediment fluctuation relative to numerous (minimum of five) fixed datum established on-site is recommended (*sensu* Fonseca et al. 1985, Merkel 1992). The number of datum should be increased to as many as practicable, covering all areas of a planting site where differences in sediment erosion and accumulation are anticipated. Readings should be taken daily if possible for at least one lunar cycle at the same time of day to allow measurements at as many tidal stages during a lunar cycle as feasible. Merkel (1992) recommended placing a one foot long 1/2-inch diameter PVC pipe halfway into the sediment as a datum and measuring sediment elevation relative to the top of the pipe. In areas of moderate waves and/or currents, we suggest that the pipe be longer and buried deeper into the sediment, up to 50 cm. We also suggest that an inverted T-shaped device for measuring sediment height be used. The cap of the T should be a 25 cm long segment of a wooden yard or meter stick bolted through the leg of the T so it can pivot when placed on the bottom. In this way the effect of local scour around the pipe on computation of sediment elevation change is minimized. These methods are designed for detection of chronic conditions; the reader should keep in mind that extreme, aperiodic events often determine limits to distribution of seagrass (*sensu* Gaines and Denny 1993).

POSSIBILITY OF NATURAL RECOLONIZATION

One question that repeatedly arises is the potential for natural recolonization and, thus, avoidance of the cost of planting. The ability of seagrass to recolonize a site is very difficult to predict. Rapid recolonization by *Z. marina* has been observed in North Carolina (Kenworthy et al. 1980, Fonseca et al. 1990) and British Columbia (Harrison 1987), as has *H. wrightii* in the Florida Keys (Thayer et al. 1994) and *Halophila* spp. (Kenworthy 1992). Annual populations of *Z. marina* in Nova Scotia, the Gulf of California, and San Francisco Bay require seeding for year-to-year persistence (in fact, planting of vegetative stock using the techniques designed to capitalize on persistent vegetative growth is wholly inappropriate for these annual populations; Fredette et al. 1985). However, it has been our observation that seedling recruitment success in some seagrasses (e.g., *Z. marina* and *T. testudinum*) have some years with extraordinary seed and seedling production. In the interim, seedling success appears to be minimal. Of course this observation could be confounded by grazing, sediment disturbance, etc., and seed production and seedling germination could exhibit little interannual variation. However, we do know that seedling recruitment to unvegetated areas varies with current regime; high current areas can have very low- to nonexistent seedling recruitment while relatively quiescent areas have heavy seed sets (Fonseca and Kenworthy 1987). Thus, there is evidence to sug-

gest that site conditions alone can influence recolonization potential (Orth et al. 1994).

Recolonization can also occur vegetatively (rhizome extension) from any adjacent seagrass. The proximity of plants and the geometry of the open area combine to produce variable recovery scenarios that are currently unquantified. However, plants close to a small (several meters wide) open area will, in the absence of seeding or fragment colonization (*sensu* Cambridge et al. 1983), colonize that area more rapidly than a larger area, a process very much like that described for patches opened in coral reefs and rocky intertidal ecosystems (Connell and Keough 1985). Bioturbation can arrest recovery of open areas (see "Bioturbation" section, above). Therefore, natural recolonization of even small-to-moderately-sized open areas can potentially be arrested, but conversely may be stimulated by the use of bioturbation exclusion devices. It is critical though, that the reason for lack of recolonization be determined. Propagule limitation and inappropriate environmental conditions (i.e., periodic exposure at low tide) can yield similar results: no coverage. We conclude that natural recolonization is almost always such a chance occurrence that is strongly influenced by disturbance events, that management practices should not, in the absence of some pilot data (e.g., monitoring of a site with planting held in abeyance or prior local quantitative observations of recolonization on similar sites), rely on natural recolonization to restore coverage.

NUTRIENT REQUIREMENTS FOR TRANSPLANTING

When considering the nutrient requirements of seagrass transplants there are three important site-specific questions that require attention. First, are there sufficient nutrients to support the growth and reproduction of transplants? Second, are nutrients present in excess of what the seagrasses can utilize, thereby available to stimulate epiphytes, phytoplankton, and macroalgal growth? Finally, are nutrient concentrations toxic to the plants? Recent evidence suggests that NO_3 may be toxic to some seagrass species (Burkholder et al. 1992). Uptake by the plants of this form of nitrogen apparently cannot be controlled, possibly leading to a loss of flexural stiffness which makes the plants lay over (leading to the same problems encountered by F. Short with *Nereis* bioturbation). In general though, there is very little additional information suggesting that any of the major macronutrients (N, P, K) occur in high enough concentration to negatively affect seagrasses. This also includes a consideration of organic herbicides in surface and ground waters, which do appear to have a negative effect on seagrasses in concentrations observed in the field (Schwarzschild et al. 1994).

There is a large body of evidence indicating that mature, well established seagrass beds can be nutrient limited under certain conditions, despite large reservoirs of nutrients in the sediments (Short and McRoy 1984, Dennison et al. 1987, Short 1987). These conditions include periods of time when optimum temperature and light regimes coincide to allow very high rates of primary production by the seagrasses (Perez et al. 1991). During these periods seagrasses extract nutrients from the available reservoirs faster than they can be regenerated by biogeochemical processes, eventually exhausting their resources (Short et al. 1985). The strongest and most consistent evidence for nutrient limitation has been demonstrated for seagrasses growing on sediments with sufficient amounts of biogenically derived carbonate to tightly adsorb phosphorus (Powell et al. 1989, Short et al.1990, Perez et al. 1991), although they may also be limited by phosphorus on siliceous sediments (Murray et al. 1992).

The largest reservoir of nutrients available for seagrasses are the sediments (Kenworthy et al. 1982, Short 1987, Fourqurean et al. 1992a). With the exception of one genus, *Phyllospadix*, all seagrasses grow rooted in soft sediments. Results from a wide range of studies, including comparisons of porewater and water column nutrient concentrations, sediment organic content, functional anatomy, and physiological ecology, all suggest that seagrasses can derive their nutrition from both the sediments and the water column (for a review, see Short 1987). However, because of relatively higher concentrations of dissolved inorganic and organic nutrients in the interstitial water, seagrasses obtain most of their macronutrients from the sediments. Fertilization experiments, which have added nutrients to the sediments, confirmed this for inorganic nitrogen and phosphorus with several North American species including *Z. marina*, *H. wrightii*, *S. filiforme*, *T. testudinum*, and *R. maritima* (Orth 1977, Orth and Moore 1982, Short 1987, Powell et. al. 1989, Short et al. 1990, Murray et al. 1992), as well as for other species such as *Cymodocea nodosa* (Perez et al. 1991) and *Heterozostera tasmanica* (Bulthuis and Woelkerling 1981). For *Phyllospadix* spp. most, if not all, of their nutritional requirements must be met by water column constituents. Because of very low nutrient concentrations in the water column, *Phyllospadix* species depend on the flux of nutrients generated by water movement and may be nutrient limited more frequently than other seagrasses (but see Koch 1993).

The nutrient requirements of some genera have received minor attention. Very little is known about the nutritional requirements of the three *Halophila* species living in the southeastern United States and Caribbean region. All three, *H. decipiens*, *H. engelmanni*, and *H. johnsonni*, grow on soft sediments, but the roots only penetrate a few centimeters into the substrate. Because rooting depths are much shallower than any of the other species, reduced access to the larger sediment reservoir may result in

nutrient limitation. Likewise, *Halophila hawaiiana*, a seagrass endemic to the Hawaiian Islands, and *Halophila ovalis*, another species common in the Pacific territories, have a morphology and rooting depth similar to the other three *Halophila* species and should also have limited access to the sediment nutrient reservoir.

Sediment reservoirs may also become depleted for species which depend on the substrate for their nutrition. This occurs when regeneration rates cannot keep up with plant demands, leading to nutrient limitation (Short 1983). Since established beds of clonally integrated plants can exhibit nutrient limitation, it is expected that young, independently (i.e., not physiologically integrated with a larger clonal unit) developing shoots of transplanted seagrasses can experience more severe limitation. Young, transplanted shoots have immature root systems, and support by translocation of nutrients from neighboring plants in the clone may be interrupted if (when) the rhizome if broken during transplanting. This is exacerbated by the fact that the rhizosphere is usually disturbed during planting. Disturbance may be more severe with bare root planting techniques than if cores are utilized. Cores usually retain the sediments intact so that the substrate is planted together with the seagrasses, minimizing disturbance to the biogeochemical recycling processes. Considering the previous discussion and a very large body of evidence indicating that seagrasses are nutrient limited, we expect that the survival and growth of seagrass transplants can be improved by addition of nitrogen and phosphorus to sediments (Kenworthy and Fonseca 1992).

Despite the evidence suggesting that seagrasses can be nutrient limited, the few studies examining nutrient fertilization of seagrass transplants have demonstrated inconsistent results (Orth and Moore 1982, Kenworthy and Fonseca 1992). Reasons for this variation include the possibility that the delivery of nitrogen and phosphorus is altered by the presence of flooded, anaerobic sediments. Most commercial fertilizers were developed for terrestrial sediments with a much smaller fraction of water to solubilize the fertilizer in proportion to a very large fraction of soil surface to adsorb the inorganic ions as they are released. Granulated fertilizers leach nutrients rapidly in flooded soils, possibly much faster than can be adsorbed or utilized by the plants. Granulated fertilizers are also difficult to deploy in a flooded sediment without a means of containing the granules. Slow release fertilizers show the most promise, although past evidence has shown variable release rate characteristics with fertilizers containing both nitrogen and phosphorus (Kenworthy and Fonseca 1992). Unfortunately, many studies using the slow release forms in field and greenhouse experiments did not directly test the release characteristics of the fertilizers nor were many of these experiments done on transplants (Pulich 1985, Short et al. 1990, Erftemeijer et al.1994). The majority of the studies were done with established or

patchy seagrass beds which may not be indicative of the response of transplants. Slow release forms of inorganic and urea nitrogen (38 percent N) and phosphorus (41 percent P), which are encapsulated separately, seem to perform more consistently and show some promise (e.g., Osmocote™). Slow release forms are more appealing because they are designed to deliver nutrients over an extended period of time in a steady dose during the most important and vulnerable time for developing transplants. Research has demonstrated the utility of slow release forms with the delivery of nitrogen and phosphorus in agar-nutrient mixtures (Perez et al. 1991, Murray et al. 1992) and improved forms of encapsulated commercial fertilizers (Fred Short, Jackson Estuarine Lab., Durham, NH, pers. com.). These studies have suggested that slow release forms will stimulate the growth and reproduction of seagrasses as well as the nutrient content of their tissue, which is a strong indication that the plants are nutrient limited and utilizing the supplemental nutrients provided by the fertilizers (Fourqurean et al. 1992b).

Originally, Kenworthy and Fonseca (1992) thought that by encapsulating the nitrogen and phosphorus separately, problems originally encountered with the lack of phosphorus release would be overcome. Subsequent work (Fonseca et al. 1994) has shown that even phosphorus alone does not always release on schedule although nitrogen pellets appear to follow manufacturers' specifications. The point here is that fertilizer additions have not always performed as anticipated based on terrestrial applications.

Kenworthy and Fonseca (1992) and Fonseca (1994) recommend that no reduction in planting effort should be enacted in anticipation of fertilizer benefits. However, they also note that no negative effects have been reported meaning fertilizer use is either neutral (considering the small additional cost per planting) or positive (accelerating new shoot formation). When employed, fertilizers should be added to the sediments at the time of planting and during the growing season of the particular species. If peat pot methods are used, a measured dose of fertilizer can be placed in the pot prior to installing the seagrass plug. If a coring technique is used, the fertilizer can be installed into the hole where the plants are to be placed after extracting the plug of sediment, or placed directly into the core that is to be planted (see "Methods" section in Chapter 3). In both cases the fertilizer may be installed into some type of porous container such as tissue paper (Bulthuis and Woelkerling 1981) or fine mesh screen (Kenworthy and Fonseca 1992). This helps avoid the problem experienced by Bulthuis et al. (1992) where bioturbation redistributed buried fertilizers onto the sediment surface, essentially terminating their effectiveness as a source of nutrition for the seagrasses. Preferably, the material containing the fertilizer should be biodegradable. Pre-weighed fertilizer can be packaged inside small paper envel-

opes, although these can disintegrate in water during handling. The envelope is stapled shut and installed with the planting units. Recommended application rates are between 5 and 10 g of balanced N-P slow release fertilizer (including the capsule) per planting unit (Kenworthy and Fonseca 1992).

For best results it may be necessary to reapply fertilizer within the prescribed release period, as long as the time is within the window of the species' growing season. The best way to determine the need for reapplication is to set aside a representative sample of fertilizer packets as controls and periodically recover replicate samples to determine the residual fertilizer, and thus, the release rate (see Kenworthy and Fonseca 1992); or simply re-fertilize in accordance with the release schedule given for the fertilizer at ambient temperatures.

Nutrients may also be added with commercially available fertilizer stakes which can be purchased at garden and hardware stores (Williams 1990). Stakes are easier to work with because the fertilizer is compacted into one single unit rather than several pellets, eliminating the need for a container. These stakes are another form of slow release but their specific delivery-rate characteristics in marine and estuarine sediments are unknown. When planting in terrigenous sediments both nitrogen and phosphorus fertilizers should be applied. In pure carbonate sediments it has appeared as though fertilization only with phosphorus (thereby avoiding the stimulation of any other macrophytes or microalgae by added nitrogen) was needed. However, Duarte et al. (1995) has shown that iron limitation may play an important role in *Thalassia* and *Syringodium* growth in carbonate sediments. We speculate that use of iron staples as anchors in planting, as recommended later, may inadvertently contribute to overcoming that limitation.

An important consideration before planting is the status of water column nutrients at a restoration or mitigation site. An excess of nutrients (from outside the planting, not from fertilizers installed in the sediment as part of the planting process) can lead to the overabundance of chlorophyll in the water column and, eventually, to severe light limitation for the seagrasses (Twilley et al. 1985, Dennison et al 1993). Excess nutrients can also lead to the growth of nuisance macroalgae which compete with seagrasses for space and light. Blooms of macroalgae may actually overgrow seagrasses and smother them (Harlin and Thorne-Miller 1981, Walker and McComb 1992, Short and Burdick in press), even the large climax species like *T. testudinum* (Tomasko and Lapointe 1991). The smaller *Halophila* species, seedlings of larger species, and young developing transplants of all seagrasses are especially vulnerable to overgrowth and displacement by macroalgae.

Nutrient enrichment in the water column and the general degradation of water quality are also responsible for stimulating the overabundance of epiphytic algae which grow on seagrass leaves (Sand Jensen and Borum 1983, Borum 1985, Silberstein et al 1986). Excessive amounts of epiphytes will shade out light and diminish the productivity and growth of seagrasses (Bulthuis and Woelkerling 1983, Wetzel and Neckles 1986, Neckles et al. 1993). Young, newly-established transplants adjusting to the shock of planting are particularly vulnerable to overgrowth of epiphytes; therefore, the nutrient status of the water column is an important consideration when selecting a planting site.

Generally, it is not practical to measure water column nutrients at the precision and frequency necessary to determine if there is a statistically significant excess present. Because inorganic nutrients in the water column are utilized and turned over so fast, an excess may not be detected by sampling the dissolved forms of these nutrients in the water column (Tomasko and Lapointe 1991). This is especially pertinent for inorganic phosphorus in subtropical-tropical systems of south Florida, Puerto Rico, the Virgin Islands and possibly the Pacific territories (although effects of volcanic soils on the sediment chemistry and seagrass growth is poorly understood) where carbonate sediments are the primary substrate (Fourqurean et al. 1992a).

Alternatively, much more practical indicators of nutrient enrichment or limitation are the organisms themselves. There are three reliable indicators that can be used as semi-quantitative descriptors of the site-specific nutrient regime. The first is the amount of phytoplankton chlorophyll which reflects the concentration of nutrients in the water column (Smith et al. 1981, Valiela et al. 1990). Sustained water column chlorophyll concentrations in excess of 10-15 mg l^{-1} are usually indicative of nutrient enrichment and a general degradation of water quality (Batiuk et al. 1992, Dennison et al. 1993, Table 1.2). At these concentrations chlorophyll can make a significant contribution to water column light attenuation (McPherson and Miller 1987, Gallegos 1994) and be detrimental to seagrass transplants.

If long-term data for dissolved inorganic nitrogen and phosphorus are available for potential planting sites located in the mid-Atlantic, Northeast, Pacific West Coast and Pacific Northwest, then the nutrient criteria provided in Dennison et al. (1993) can be used to accept or reject a location. These criteria would apply mainly to sites with terrigenous sediments and not with biogenically derived carbonates. Dennison et al. (1993) showed that polyhaline regions of the Chesapeake Bay supported *Z. marina* where dissolved inorganic nitrogen and phosphorus were < 0.10 and < 0.67 uM, respectively. These are median values derived from data obtained over several years, but only for the growing season of the plants (March to November). At these

same locations median values for chlorophyll a were < 15 ug l^{-1}. Keep in mind that these criteria were developed using established seagrass beds that persisted over multiple growing seasons and define upper threshold values.

The success of newly developing plantings could potentially be improved by avoiding sites with similar maximum concentrations as those described by Dennison et al. (1993). We suggest that, as a first guess, locations with concentration values ~25 percent lower than the predicted maximum constitute a reasonable starting point to introduce plantings. These criteria (Table 1.2) are probably reasonable for planting sites in the northern Gulf of Mexico and Texas, but are probably much too high for comparatively oligotrophic conditions of southeast Florida, Florida Bay, Florida Keys, Puerto Rico, the Virgin Islands, and the Pacific Territories. In these oligotrophic waters with carbonate sediments, larger amounts of nitrogen and phosphorus are normally tied up in plant biomass and sediments. Water column concentrations of nitrogen and phosphorus in the ranges reported by Dennison et al. (1993) would result in the relaxation of nutrient limitation for phytoplankton and benthic macroalgae, likely leading to eutrophication (Tomasko and Lapointe 1991). Unfortunately, there are no comprehensive studies that define nutrient criteria as specific as Dennison et al. (1993) for the aforementioned carbonate environments. We recommend that inorganic phosphorus criteria for suitable planting sites in areas of carbonate sediments be established at concentrations an order of magnitude less than reported by Dennison et al. (1993) and Tomasko and Lapointe (1991).

A second indicator of nutrient effects is the tissue Redfield Ratio or C–N–P content of the seagrasses themselves (Atkinson and Smith 1983, Short 1987, Duarte 1990, Fourqurean et al. 1992b, Perez et al. 1994). If seagrasses are present in the vicinity of a planting site, evaluation of their tissue nutrient composition can be used to determine the necessity for fertilization or provide some indication that there are excess nutrients. In nutrient enriched sites seagrasses have higher than average tissue concentrations for the nutrient occurring in extra abundance relative to the composition of plants at sites isolated from enrichment (controls). Deviation in the tissue concentration of a particular element can provide a clue as to which nutrient is either limiting or in excess. Duarte (1990) suggested that seagrass leaves with median nutrient levels of <1.8 percent N and <0.2 percent P are strongly nutrient limited. If seagrasses in the area at or near the planting site display similar levels, fertilization of transplants could be helpful. Likewise, deviations upwards from these values would indicate either adequate nutrients or, possibly, nutrient enrichment at the site. The advantage of using the plant tissue is that the seagrasses act as a barometer for continuous longer-term monitoring of their environment and reveal the conditions without sampling error (Dennison et al. 1993).

A third indicator of nutrient enrichment is the presence of large amounts of macroalgae, especially the faster growing species of green algae like *Ulva* spp. or *Enteromorpha* spp. These non-vascular plants utilize nutrients at a much faster rate and have higher turnover than seagrasses, allowing them to out-compete vascular plants for essential nutrients (Harlin and Thorne-Miller 1981, Lapointe and Clark 1992, Walker and McComb 1992). There are no quantitative criteria defining a threshold amount of macroalgae that is detrimental to seagrasses, but if quadrat sampling indicates macroalgal cover in excess of 50 percent of the bottom it is likely that seagrasses will be negatively impacted.

LIGHT REQUIREMENTS FOR TRANSPLANTING

All seagrasses in the United States with the exception of one genus, *Phyllospadix*, grow in flooded and chronically anoxic sediments. This section focuses on those seagrasses in unconsolidated sediments given to anoxia. Light requirements for the intertidal *Phyllospadix* spp. are unknown, but their distribution is likely more tied to emersion limitations (see "Emersion Effects" above).

To survive and grow in anaerobic sediments, seagrasses require photosynthetically-produced O_2 to support the aerobic metabolism of non-photosynthetic root and rhizome tissues and the dark respiration of leaves (Smith et al. 1988b). In the absence of available O_2, less efficient anaerobic fermentation leads to a demand for carbohydrate reserves which may be provided by stored material in the rhizome or translocated from healthy adjoining shoots in the clone (Harrison 1978, Dawes et al. 1987, Libes and Boudouresque 1987, Tomasko and Dawes 1989). Transplanting may disrupt the physiological integration between rhizome and adjoining shoots, limiting the ability of healthy shoots to support stressed short shoots (ramets), depending on the amount of physiological integration among shoots in the first place (a phenomenon that is now only poorly understood). In mature, well established meadows, neighboring shoots attached by the same rhizome may contribute to the survival and growth of other shoots by translocating carbon and nutrients which can be utilized during periods of time when resources are depleted. This relationship can be especially critical for young, developing plants that have not yet produced enough photosynthetic tissue to be independent of clonal support. Young developing shoots are very important when transplanting because they are the basis for survival and expansion of the planting. Therefore, if young shoots are physiologically dependent on adjacent, older shoots, the light requirements of transplanted seagrasses require special attention because they are likely to be higher than the requirements determined from established meadows. Moreover, there are cases in terrestrial systems where

young ramets are sacrificed by older ramets should those young ones venture into physiologically unsuitable areas. Even if this were the case in seagrass, most planting stock consists of fragments of the root-shoot-rhizome complex and the integration of shoots is broken in any event. Therefore, even if young shoots were to be sacrificed by older ones, the feedback mechanism would not exist when rhizomes are fragmented during planting, therefore light requirements should still be higher for the young shoots.

When transplanting with mature shoots, either completely removed from the sediment (bare root technique) or left intact in a sediment core (plug), the rhizomes are severed. Presumably, the plug method would be less stressful because of minimal disturbance to the rhizosphere. However, in both cases transplant survival and new growth depend on the formation of vegetatively reproduced shoots which may have less support available than shoots growing in a dense, clonally-integrated meadow.

Seagrass minimum light requirements have been determined by three approaches: (1) photosynthetic measurements, (2) whole plant carbon balance models, and (3) correspondence (correlation analysis) between light availability and maximum depth of seagrass growth. Photosynthetic measurements alone are inadequate and have severely underestimated seagrass light requirements (Drew 1979). Carbon balance models have improved our understanding of light requirements because they account for the additional carbon requirements of non-photosynthetic tissue (Zimmerman et al. 1989, Fourqurean and Zieman 1991, Zimmermen et al. 1994). Correspondence analysis between some statistical average light level (mean or median) and the maximum depth to which seagrasses grow uses a long term response by the plants to record the requirement (Dennison 1987, Duarte 1991, Kenworthy and Haunert 1991, Dennison et al. 1993, Onuf 1994). The third approach is particularly useful because it depends on the plants interacting with their environment to reveal their actual response, which has usually indicated a higher light requirement than either of the first two approaches (Kenworthy and Haunert 1991). Even though a correspondence analysis seems more appropriate to estimate light requirements, it still must be adjusted upward for estimating transplanting requirements. The deep edges of the beds used as the barometer in the correlation between light and depth of growth are formed from well-established stands and are probably maintained by support from adjoining shoots. Moreover, the level of resolution in a correspondence analysis does not contain enough information about the possible influence of short-term departures from average light levels (Zimmerman et al. 1994). The survival of individual shoots in a transplanting unit is thus potentially vulnerable to short term fluctuations in light levels whereas mature beds are buffeted by reserves within the clone. For these reasons we recommend that the light environment necessary for the

initial survival and growth of transplanted seagrasses exceeds minimum requirements for established meadows.

Another important consideration is the fact that light requirements are not the same for all species of seagrass. Large differences occur between genera that are based on growth form of individual shoots, clonal architecture, and physiology. In temperate regions, both in the Northeast and on the West Coast, the dominant seagrass, *Z. marina*, has a much different growth architecture than any of the five species found in the southeastern United States. Initially, on *Z. marina*, vegetatively reproduced short shoots are arranged morphologically and physiologically close to the parent shoot from which they are formed. This relationship is only temporary because both the parent shoot and its offspring grow away from each other quite rapidly. Horizontal rhizome growth separates the mature plant from the younger shoot and the metabolic activity of the rhizome diminishes rapidly as the older nodes age, severing the physiological coupling between ramets in the clone (Kraemer and Alberte 1993). *R. maritima*, *Z. noltii*, and *Z. japonica* all have a growth architecture and morphology similar to *Z. marina* and should be as sensitive to light-limiting conditions immediately following planting.

In the southeastern ecoregion, the potential for integrating shoots is greater for the three larger and most common subtropical genera, *T. testudinum*, *S. filiforme*, and *H. wrightii*. For these species horizontal internode distance is deterministic and adjoining short shoots remain the same distance from one another, usually throughout their entire life span. For all these species, adjoining shoots in a clone have far greater potential for sharing resources and supporting one another than is available for *Z. marina*. In *Zostera*, horizontal rhizome growth and new shoot formation are not as closely coupled and, therefore, clonal integration does not likely make as great a contribution to survival. The individual *Zostera* shoot in the initial stages of planting is independent of the clone sooner and may be more vulnerable to physiological stress than either of the three larger subtropical species.

The fourth tropical genus and the smallest of all seagrasses, *Halophila*, appears to have the lowest light requirement. *Halophila decipiens* is usually found growing in the deepest and most turbid water and is almost never observed in the canopy of the larger species; these are attributes that make its detection by conventional remote-sensing methods unlikely (Dobson et al. 1995). Because it has thin leaves and relatively lower root rhizome biomass it was once believed to have been better equipped to survive in low light environments (Josselyn et al. 1986). But this conclusion was drawn without full consideration of the life history of the plants and the seasonal variation in light. Many coastal environments have regular seasonal fluctuations in

light which include periods when light is well above and well below the average. Part of the explanation for growing in low light environments is that *H. decipiens* reproduces by seed and avoids the stress of the lowest light periods (usually winter) in a temporary seed bank (Kenworthy 1992). *Halophila engelmanni* has a similar life history strategy, but unlike *H. decipiens*, this species may be found beneath the canopy of the larger seagrasses. Very little is known about the life history or physiology of *H. johnsonii*, however, it does grow in high light environments in the intertidal zone (Kenworthy 1992). Also, little is known about how these three *Halophila* species would respond to transplanting. However, we have successfully transplanted *H. decipiens* among existing beds of the same species in 15 m of water on St. Croix, USVI, but we are unaware of any other plantings with this genera.

The ability to avoid the stress of low light periods by surviving in a seed bank may be important for the persistence of other tropical genera (McMillan and Phillips 1979) and temperate species *Zostera marina*, *Z. noltii*, and *Ruppia maritima* as well (Harrison 1982, Orth and Moore 1983, Hootsmans et al. 1987). Like *H. decipiens*, some temperate species may have annual life history strategies completely dependent on seed reproduction for their survival (Keddy and Patriquin 1978, McMillan 1983). In these annual populations the plants do not experience a large portion of the seasonal light regime. Even though correspondence analysis has demonstrated higher light requirements for seagrasses in general, the averaging processes may include periods of low light that are not actually critical to the survival of the plants. In tropical, subtropical, and temperate regions seagrasses reduce their growth rates significantly during the colder temperatures in winter, and therefore, the light regime during this period may be much less important to the plants.

Light requirements should be determined based on the time period when the plants are responding to solar insolation and not when metabolism is slowed or when the population is residing in the sediments as seeds. This suggests that seagrass minimum light requirements determined from correspondence analysis (Dennison 1987, Duarte 1991) exceed the frequently cited value of 10 percent surface light. Recent evidence suggests that more realistic values for *H. wrightii* and *S. filiforme* are in excess of 15-20 percent (Kenworthy 1992, Kenworthy and Fonseca 1996), and for planting may even be higher (~25 percent). Moreover, light requirements of an individual species may vary as a function of the optical water quality indicating a very site specific component (Kenworthy 1992, Gallegos 1994, Gallegos and Kenworthy 1996).

Pre-restoration monitoring and site selection criteria should incorporate elements of the preceding discussion to improve the likelihood of planting success. Most sites will not have long-term data bases from which to characterize the light

environment. If they do, then only data collected during the growing season should be used to calculate the appropriate statistic for a light level parameter. Regional growing seasons (see below: Planting Contingencies by Ecological Region) can be determined from the literature and planting should occur as early as possible in the season to take advantage of the optimum light regime.

Without light data taken over a growing season, the next best parameter is to utilize the maximum depth to which seagrasses grow in seagrass beds located in the area around the planting site. These reference beds can only be used to establish a maximum depth for transplanting rather than a specific light level. The reference site should have as similar a fetch (unobstructed over-water distance over which the wind blows), sediment composition, and tidal velocities as the planting site to assure that suspended sediments and light attenuation by turbidity at the two sites will be similar. Also, there should be as little difference as possible in freshwater discharge and water column chlorophyll concentration so that there are no gross differences in water color which could mean a different level of light attenuation. The reader should keep in mind that only light recordings performed at high frequency during growing seasons can yield prediction strength.

The depth to which local seagrasses grow would represent the minimum for an established bed and would likely overestimate the depth that transplants could survive. Beers Law describes the decline of photosynthetically-active radiation (PAR: the wavelengths of the light spectrum that activate chlorophyll) with depth in the water column by the following equation:

$$Iz = I_o * e^{-kz}$$

where:
I_o = Incident light just beneath the surface
Iz = Percent incident light at depth
e = base e
$-k$ = diffuse light attenuation coefficient
z = water depth

Therefore, we can estimate the percent light reaching a predetermined depth. If seagrasses in the area grow to a depth of 2.0 m and the average diffuse light attenuation coefficient is 0.75 (similar to the Indian River Lagoon; Kenworthy 1992), this indicates seagrasses require 22 percent surface light at their maximum depth of growth. At this location the light reaching shallower depths of 1.75 or 1.55 m is 26 and 32 percent, respectively. Transplanting at these two shallower depths would

increase the relative amount of light available by 18 and 45 percent, respectively (+4 and 10 percent, respectively, of the amount of light available at the lower limit). Because the decay of light in water is exponential in the upper portion of the water column, a small change in depth yields a proportionally larger change in percent surface light reaching that depth. Reducing the depth of planting by 12.5 percent increases the amount of light by 18 percent whereas a reduction in depth by 25 percent adds 45 percent more light. This also works in the other direction whereby planting at slightly deeper depth would yield considerably less light. Paying close attention to depth and its relationship to available light could make the difference between success and failure of a planting.

In some cases planting sites may be isolated from established reference beds, especially in large-scale restoration efforts where entire lagoons or large portions of estuaries are involved. In these instances reference sites with established seagrass beds must be carefully matched with the restoration site for sediment composition, fetch, and tidal velocities. Short-term between site-paired comparisons of optical conditions can be made *in lieu* of a direct correspondence analysis or the availability of any long-term water quality data in order to grossly infer the lower depth limit. A paired comparison between sites would involve the acquisition of either light or water quality data over the same time period and under the same environmental conditions at each site (e.g., no localized storms affecting one site and not the other). Measuring equipment and methods must be intercalibrated to avoid detecting differences that cannot attributed to the sites.

One of the best parameters for inter-site comparison is the diffuse attenuation coefficient for photosynthetically active radiation (k_d PAR). If available, this parameter can be used to calculate percent surface light reaching a predetermined depth in the equation for Beers Law (p. 94) so that light available at depth can be used to help select planting depth. If at all possible, the technique for estimating k_dPAR should utilize quantum sensors instead of a Secchi disk (Kenworthy 1992). A Secchi disk severely underestimates light attenuation in estuarine water where there are dissolved organics (influencing color). Two types of quantum sensors are available, the cosine-corrected flat type sensor that measures downwelling PAR and the spherical quantum sensor that measures light from all directions (scalar PAR). The spherical quantum sensor is the preferred equipment although under most conditions the two different sensors will yield similar attenuation coefficients.

In a short-term paired-site comparison the sensors can be deployed in either a continuous or profiling configuration. In a continuous mode, at each site where one

intends to plant seagrass, two sensors should be placed in the water column at two fixed depths at least 20–50 cm apart and PAR recorded continuously using a data logger (Zimmerman et al. 1994). A calculation of k_dPAR at each site over the same time period should be made. Attenuation coefficients should be calculated for time periods between 10 a.m. and 2 p.m. to avoid the errors associated with solar angle and path length (Miller and McPherson 1995). If the equipment is available, this method is appropriate for small sites, but for larger sites, where intra-site variation may be a problem, a profiling method is necessary. This method uses two sensors deployed at fixed depths from a mobile vessel so that more stations can be sampled to examine spatial variation. The same calculation algorithm is used as described above, however, for inter-site comparison one must be certain that profiles are obtained at the same times for accurate comparisons. Ideally, both the continuous recording and the profiling methods should use a base station with a sensor measuring incident radiation to correct for errors from local cloud conditions (Morris and Tomasko 1993).

Another method to compare sites is to use a properly calibrated optical water quality model (Morris and Tomasko 1993, Gallegos 1994, Gallegos and Kenworthy 1996). This method estimates light attenuation by summing the additive properties of scattering and absorption due to three commonly measured water quality constituents: turbidity (NTU), color (Pt-Co units), and chlorophyll (chl in mg l^{-1}). This kind of modeling approach is convenient because once the calibration is completed the model uses the water quality constituents that are inherent optical properties and not subject to errors of solar angle (i.e., time of day). Thus, measurements can be taken at any time of day and the information tells you what particular water quality constituent is having the greatest effect on light attenuation. Model calibration requires a minimum of 30 profiles of these 3 constituents and light attenuation for a water body. Calibrations have been successfully completed in the Chesapeake Bay region (Gallegos 1994) and the Indian River Lagoon (Gallegos and Kenworthy 1996).

SALINITY AND TEMPERATURE REQUIREMENTS FOR TRANSPLANTING

Salinity and temperature tolerances of seagrass species must be considered when selecting off-site planting locations. Seagrasses exhibit a wide range of tolerances to salinity but the effect of periodicity and duration of extremes in salinity on seagrass survival are poorly documented (see review by Zieman and Zieman 1989). Matching salinity regimes between the planting site and donor site is therefore strongly recommended. Temperature regimes should be similar as well. Temperature extremes

may be problematic if a planting site has been constructed with restricted circulation allowing water temperatures to rise above levels found in natural beds.

Known temperature and salinity tolerances and optimum ranges for seagrasses growing in the continental United States are presented in Table 2.1. It is important to realize that the stress effects of these variables may be synergistic, but the effects of any such synergism on planting survival are poorly known in nature and likely vary widely depending on circulation, tidal zone, and geographic location within a species' distribution.

As seen in the data presented in Table 2.1, the tropical seagrasses are more stenothermic than temperate seagrass species. *Z. marina* has the widest temperature range (-6.0 to 40.5° C), and is found growing in a variety of temperate to sub-arctic habitats on both the east and west coasts of the United States. The range of reported optimal temperatures for this species is also greater than that reported for any other species (Bieble and McRoy 1971, Phillips 1984, Thayer et al. 1984, Evans et al. 1986, Bulthuis 1987). Data concerning the introduced species, *Z. japonica*, is less readily available, but its distribution suggests that its temperature tolerances are close to, if not greater than, that of *Z. marina* (Baldwin and Lavvon 1994). *R. maritima* and *H. wrightii* also have broad temperature tolerances as witnessed by their distribution patterns along the east Coast. Both are wide ranging species, often found in estuarine conditions where temperature conditions vary greatly with water depth, circulation patterns and exposure to periods of desiccation. While these species are often found growing in mixed beds with *Z. marina*, they have higher optimum temperatures (Evans et al. 1986, Orth and Nowak 1990, Zieman 1982a, Zieman and Zieman 1989).

Seagrasses growing in the more stenothermal subtropical and tropical marine conditions have narrower temperature tolerances than the estuarine species listed above (e.g., *T. testudinum*, *S. filiforme*, *Halophila* spp.). Information regarding members of the *Phyllospadix* genus is sparse. The distributional patterns of the three United States representatives of this genus suggest that they have similar temperature ranges, with a low end temperature of 5 and a maximum around 25° C (Drysdale and Barbour 1975). Tropical seagrass species are the least temperature tolerant, with maximum ranges of 15 degrees and optimum temperatures close to 30° C (McMilllan and Phillips 1979, McMillan 1984, Zieman 1982a, Zieman and Zieman 1989).

Salinity tolerances of seagrasses follow a similar pattern with *R. maritima* and *Z. marina* having the broadest tolerances. The coastal and marine species, which live in less euryhaline conditions, are more sensitive to changes in salinity. As with other

physico–chemical requirements, comparisons of temperature and salinity with existing beds provide the best local guidance for placement of planting areas. In the absence of nearby vegetation, a minimum of weekly monitoring during both ebb and flooding tides should be conducted on a projected planting site to determine if values fall within those proscribed in Table 2.1. More intense sampling should be conducted (daily) just after several rainfall events, hopefully of differing rainfall amounts, to determine the response of a site to freshwater inflow. Although the tolerance ranges are known, the effects of dosing periodicity of either salinity or temperature extremes on planting survival (or natural beds for that matter) are unknown to us. For want of better guidance, we suggest that persistence of borderline values for more than five days should be cause for concern about site suitability.

Table 2.1. Temperature and salinity ranges of seagrass species occuring in the continental United States, optimal ranges are in parantheses.

Species	Temperature Range	Salinity Range
	(° C)	(ppt)
Halodule wrightii	9 – 37 (20–30) i,l,m	3.5 – 44 (20–35) i,l,m
Halophila decipiens	25 – 30 (20–36) d,j	30 – 35 (?) d,j
engelmannii	15 – 30 (24–28) c,j	15 – 35 (?) c,j
johnsonii	15 – 30 (?) d	20 – 30 (?) d
Phyllospadix torreyi	5 – 25 (11–21) e	2.9 – 29 (25–30) e
scouleri	?	?
Ruppia maritima	7 – 35 (20–30) f,j	0 – 32 (0–15) g,j
Syringodium filiforme	20 – 35 (30) i,j	20 – 35 (25–35) j
Thalassia testudinum	20 – 35 (25–32) b,h,i,l	3.5 – 60 (25–35) l,m
Zostera marina	0 – 40 (5–20) b,f,k	0 – 35 (?) k
japonica	5 – 30 (?) a	10 – 30 (26) a,e

a. Baldwin and Lavvon 1994
b. Bulthuis 1987
c. Dawes et al. 1987
d. Dawes et al. 1989
e. Drysdale and Barbour 1975
f. Evans et al. 1986
g. Mayer and Iow 1970

h. McMillan and Phillips 1979
i. McMillan 1984
j. Phillips 1960
k. Thayer et al. 1984
l. Zieman 1982a
m. Zieman and Zieman 1989

MICROPROPAGATION AND LABORATORY CULTURE OF SEAGRASS FOR PLANTING

Laboratory culture of plant fragments (micropropagation) for large-scale field plantings is an active research area (Durako and Moffler 1981, 1984, Lewis 1987, 1990, Ailstock et al. 1991, Koch and Durako 1991, Durako et al. 1993, Bird et al. 1994, DeLeon et al. in press.). In the future, large-scale plantings may rely on laboratory-reared plants as there are many potential advantages to using this approach. However, there are many practical problems that must be overcome first as well as questions regarding the general efficacy of the approach. Below we contrast some of the argued benefits with what we feel are limitations to the technology. In summary, the advantages of relying on laboratory-cultured plants are as follows:

1. **Donor bed damage reduction:** Reduce damage to donor bed by making small field collections and geometrically expanding the numbers of plants in the lab to provide planting stock;

2. **Genetic stock improvement:** Improve genetic mix of stocks (avoidance of founder effects, see section "Pre-Project Planning Considerations," above) to go into the field;

3. **Disease/stress resistance:** Select for disease-resistant or stress-tolerant strains of plants;

4. **Cost reduction:** Reduce project costs through mass production of planting units;

5. **Stock availability:** Maintain donor stocks to meet the sporadic demands of disjunct planting projects;

6. **Bioassay tool:** Develop genetically consistent stocks that can be deployed and thus used as a bioassay standard of water quality, and potentially, planting-site suitability for subsequent restoration projects.

Taken together, the above arguments would seem to logically place culture techniques at the forefront of the seagrass mitigation/restoration effort. However, there are serious questions regarding many of the proposed benefits of micropropagation. The disadvantagess to the above claims are as follows (response by corresponding number):

1. **Donor bed damage reduction:** Sustained injury to donor sites from one-time impacts have been demonstrated to be an issue for only one of the ~13 North American seagrass species, *Thalassia testudinum* (e.g., Zieman 1976, Durako et al. 1992; see following section). Donor bed injury recovery can be rapid (Fonseca et al. 1994) especially if a minimum attempt is made to disperse the collection effort. However, some States (e.g., Florida) have placed restrictions on harvest of wild seagrass stock for planting projects; this appears to fuel the donor-bed impact argument.

2. **Genetic stock improvement:** "Improvement" of genetic stocks is currently speculative. A broad knowledge of the existing genetic structure of seagrass populations and the factors influencing genetic stock (especially site-specific; *sensu* Ruckelshaus 1994) would be requisite to form a comparative basis for "improvement," which requires stricter definition. Moreover, while strain selection is applicable to terrestrial crops where gene flow and population interactions are not an issue, the potential for improvement of genetic structure is untested in the management of wild, clonal plant communities.

3. **Disease/stress resistance:** The disease/stress resistance of micropropagated plants has not yet been demonstrated, nor has the consequences of trading off natural genetic diversity for selection of, and introduction to nature, of a few strains designed to meet specific environmental problems. Moreover, if a bed were developed in a stressed area through selection of resistant stock, would such a bed become functionally equivalent to natural beds? Introduction of manipulated genotypes remains a significant issue in population ecology, and is many years away from resolution.

4. **Cost reduction:** While large-scale production costs would be less on a planting unit basis, only DeLeon et al. (in press) has, to our knowledge, considered a quantitative review of the entire planting cost using micropropagation techniques that includes the overhead and amortization of culturing facilities, materials, and labor. Break-even points (i.e., number of planting units needed) have not been determined. Moreover, culturing does not eliminate out-planting costs which may be similar among cultured or wild-harvested plantings.

5. **Stock availability:** Like (4) above, we are not aware of published data on cost break-even points, which are strongly influenced by required frequency and magnitudes of requests for planting stock needed to support such a facility.

6. **Bioassay tool:** This aspect of seagrass micropropagation offers one of the most powerful applications of this technology. Having a "standard plant," coupled with plant health assessment techniques such as variable fluorescence techniques (*sensu* Adams and Bate 1994) could yield rapid and consistent water quality and planting site suitability indices, as evidenced by Durako et al. (in press).

At this time, there are only two seagrass species that have been successfully cultured: *Ruppia maritima* and *Halophila decipiens* (M. Durako, University of North Carolina, Wilmington, pers. com.), two species that have very high sexual and asexual reproduction and thus, high colonization (and likely, donor bed recovery) rates. The technology is not currently available to the only species for which there are demonstrable, long-term donor bed impacts, *T. testudinum*.

WILD STOCK SELECTION, AVAILABILITY, AND PERFORMANCE

The choice of species is often dictated by project goals, such as the desire to replace in kind the seagrass species that was lost. In subtropical areas where several species co-occur, it is sometimes appropriate to temporarily substitute faster-covering species in order to stabilize a planting site (*sensu* "compressed succession," attributed to M. Moffler). The specific choices of available species are covered under each ecoregional below. In general however, the early recommendations of Addy (1947) still hold where matching conditions at donor and planting sites were recommended.

For seagrass planting projects to eventually be successful, it is critical that they consider physiological requirements and life histories when selecting a planting site (see sections dealing with growth requirements, e.g., light, nutrients, temperature, salinity, above). For example, species with a slow coverage rate (i.e., *T. testudinum*) are very difficult to restore in the time frame often allotted projects. It can take decades for a bed to re-create the dense root system, organic-rich substrate, and nutrient cycling capabilities of turtlegrass beds (*sensu* Zieman 1976). *Halophila* spp. have very different strategies. This species often colonizes disturbed areas rapidly and requires relatively little light to grow (Josselyn et al. 1986). Interestingly, although the spatial distribution of *Halophila* spp. indicates an ability to colonize low light environments relative to other seagrasses, it only has growth during times of the year when light levels are at their highest. This signals a potentially complex interaction between light and temperature in effecting *Halophila* distribution. An individual leaf pair of *H.*
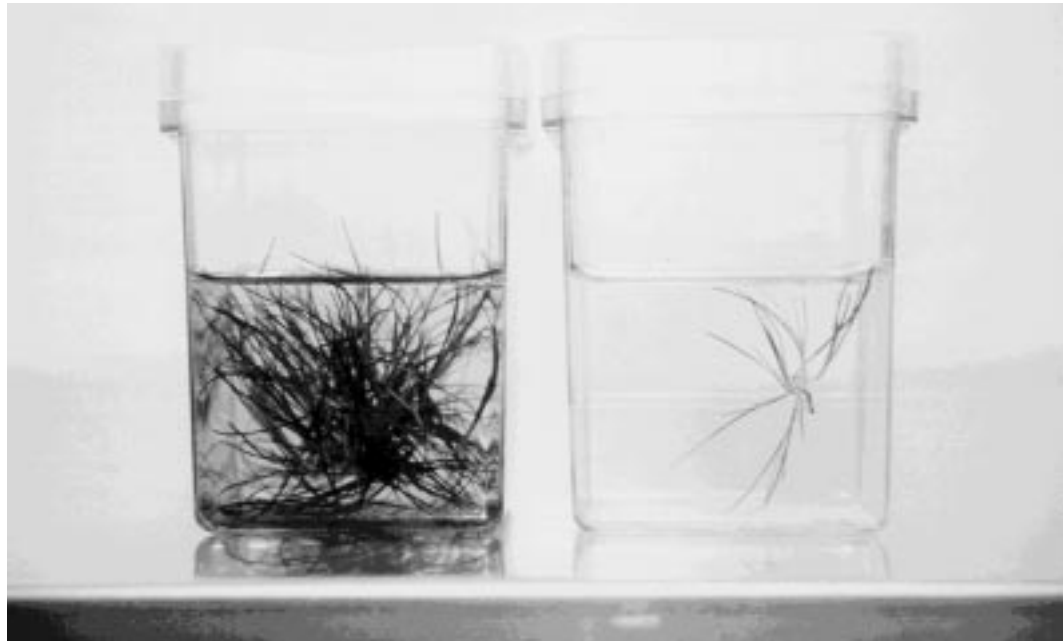
Figure 2.6. Ruppia maritima *in culture. Courtesy M. Durako.*

decipiens may live for only six weeks, and produce many seeds, a strategy typical of a species living in marginal environments.   Its shallow root system, however, makes it vulnerable to disturbance. *Ruppia maritima* (widgeon grass) has a wide tolerance of salinities and grows in fresh water, brackish water, or among other seagrasses in full strength seawater. Like *Halophila*, this species has a very high seed production and covers the bottom quickly. Finally, *H. wrightii, S. filiforme, Zostera marina* and, likely, *Z. japonica* have comparatively moderate coverage rates. Little is known regarding coverage rates of other seagrass species in the United States, such as *Phyllospadix* spp., under planting conditions. For the subtropical species, the comparative coverage rates are important both for predicting recovery rates (e.g., Fonseca et al. 1987a,c, Lewis 1987) and choosing a fast-covering species with which to initiate plantings (i.e., "compressed succession": begins with shoalgrass, allow coalescence, and then add the slower-growing target species if it were initially different from shoalgrass). For temperate areas where the pioneering species is the same as the climax species (i.e., begin with eelgrass and end with eelgrass), coverage rates are useful for recovery rate estimation. Thus, the different growth strategies of seagrasses implicitly define the anticipated performance, monitoring, planting schedule and, ultimately, function of the restored system.

Although some data exist to select planting stock by ecotypes (Backman 1991), costs of collection are also important. Collection of plants from areas of high densi-

ty, such as sandy shoals, is often more cost-effective when plants are small. Larger individual plants, such as *Zostera*, that are sometimes planted singly or with only a few in a planting unit (Davis and Short 1997), are sometimes removed more easily from softer sediments. Some seagrasses, such as *Halodule* and *Syringodium*, sometimes have ramets extending, rootless, into the water column, and these make excellent planting stock without having to extract material from the donor bed rhizosphere (Fonseca et al. 1987a,c, Lewis 1987). *Thalassia*, with its deeper rhizome system, is most easily collected at bed margins where the rhizome development is nearest the sediment surface.

At this time virtually all planting material must be obtained from existing, wild vegetative stocks. However, the collection of vegetative material from existing beds is rigorously managed in many states. Collection without appropriate permits may result in fines. Because of the evolving nature of this field of restoration science, it is imperative that anyone planning seagrass planting carefully coordinate their actions with state and local government. It is not uncommon to find permits required from not only the federal government, but also numerous state agencies. Individual counties and municipalities too may require consultation. Because of the increasing volume of permit requests reaching permitting agencies, obtaining a permit could take months. Such a delay must be anticipated in order to collect plants (if approved) and plant at the desired time of the year.

Once planting is permitted, wild stocks are usually used. *Zostera marina*, *H. wrightii* and *S. filiforme* can be harvested from wild stands with no long-term (> 1 year) impact to the donor site (Williams 1990, Fonseca et al. 1994) (Figure 2.7). However, **unless specifically created as a donor site, repeated harvest of donor sites within a calendar year should not be permitted.** It should also be noted that wild stock harvesting will cause some interim loss of habitat functions and productivity. Therefore, we recommend that harvesting impacts be composed of numerous, individual small collections rather than opening large holes in the seagrass cover.

Although not currently documented, it is highly probable that *Ruppia*, *Halophila* spp., and other *Zostera* spp. would recolonize small harvest patches quickly (< 0.25 m² patches returning to normal density within 1 year) because of their high population growth rate and seed production. Harvest from high current areas (> ~30 cm/sec) however, could initiate the development of an erosion scarp which would spread and damage the donor bed irreparably (*sensu* Patriquin 1975). *Thalassia* can be transplanted with good survival but slow population growth (Fonseca et al. 1987a,c, 1989a, Lewis 1987, Tomasko et al. 1991), but harvest damage to those donor beds may last for years (Zieman 1976, Fonseca et al. 1987c), and harvest of vegetative
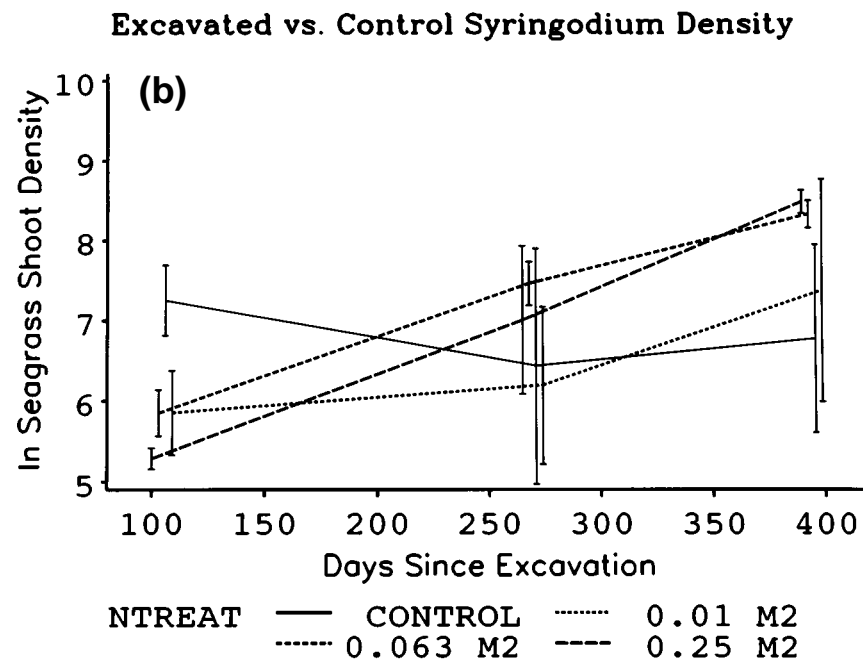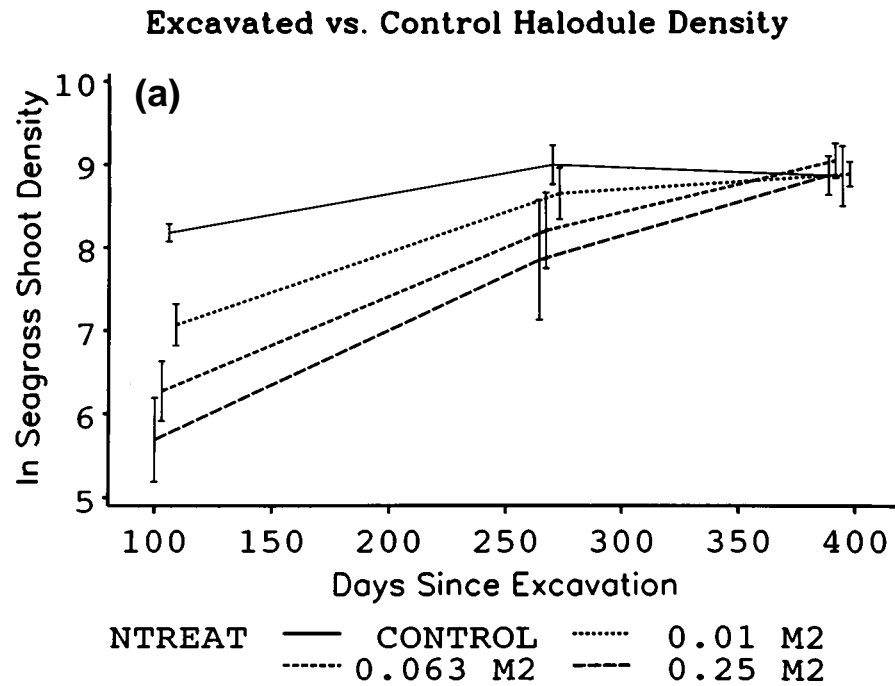
Excavated vs. Control Halodule Density

(a)

Excavated vs. Control Syringodium Density

(b)

Figure 2.7. Recovery of donor beds harvested for planting Halodule wrightii *(a) and* Syringodium filiforme *(b). Shoot density* m^{-2} *by original excavation size and time since excavation (days). C=unexcavated control. NTREAT=treatment, or size of excavation (M2 = m^2); control=no excavation. Taken from Fonseca et al. 1994.*

Thalassia stock should be from bed margins (to minimize rhizosphere disturbance) if a salvage operation (from planned or permitted disturbance) is not available. Another means of acquiring *Thalassia* planting stock is to harvest its seeds which wash up on shore (Lewis and Phillips 1980), which has no negative influence on existing beds. The impact of harvest of seeds or seedlings of any species from within existing beds or colonizing areas currently has an unknown effect on the maintenance of seagrass in those areas. However, given that seed harvest probably gathers only a small percentage of the seed production, we expect the impact to be small. The impact of donor bed harvesting on *Phyllospadix* spp. is largely unknown.

In general, planting stock should be selected from a site with conditions as similar as possible to the planting site, as near the planting site as possible, and at similar or equal water depths, salinity, and sediment type. The concept of choosing plants of the same size as those lost, perhaps accounting for potential races of seagrass, was suggested 45 years ago (Addy 1947). Little data have emerged to suggest changing this practice although concerns have been voiced regarding the maintenance of genetic diversity in "Pre-Project Planning Considerations," above). Until more is known about the genetic structure of seagrass ecosystems, harvesting of plants from as wide a geographic range as feasible is recommended. Similarly, matching sediment types of the donor site with the planting site (percent silt and clay, and percent organic matter content of the sediment) is thought to facilitate transplant success.

Planting material may become available as salvage prior to the imposition of a project. Utilization of salvaged material requires good up-front organization so that a planting site is available before the plants are destroyed (e.g., turtlegrass, Lewis 1987). Long-term storage of salvaged plant material to use for future plantings has not be scientifically evaluated, but has been accomplished for at least a week (pers. obs.). Longer term storage may be possible but may significantly increase handling costs.

In summary then, by use of environmental monitoring data, the most prudent way to select planting stock is to match conditions at a donor site with the planting site. The temperature, salinity, surface sediment (top 3 cm) particle size and organic content, tidal current speeds and wave exposure of the planting should be as similar as possible to that of a donor bed of the same seagrass species.

Long-Term Management

Creation of transplanted beds for the sole purpose of providing donor material to subsequent operations would be prudent. This would alleviate the problems of stor-

age costs, relieve some of the time constraints and permitting problems that accompany most projects, and prevent damage to native seagrass beds. However, once these beds are planted, they fall under the permit jurisdiction of resource agencies as would any seagrass bed. However, experimental beds, such as those we have created, total many acres and were not created to offset any particular loss. These should be made available as mitigation/restoration donor site. In any event, planting of beds for future donor material needs to be organized early and in coordination with permitting agencies. Moreover, to avoid net losses in baseline acreage, we recommend that planted beds be given special status and be protected from any subsequent consideration for permitted impacts. Given that early stage plantings have been found to have lowered genetic diversity (see above section), institution of these donor sites should be linked to an evaluation of their genetic structure to avoid embedding additional lowered genetic diversity into planted populations.

PLANTING CONTINGENCIES BY ECOLOGICAL REGION

Northeast Region — Maine through New Jersey: known species present = *Zostera marina* and *Ruppia maritima*.

Compared to many other parts of the country, the growing season is shorter here. Water temperatures are also comparatively cold and will be a cost factor in planting operations especially when using divers. Planting may begin as early as the waters are ice-free, but to obtain robust planting stock one usually will have to wait until April or May, and sometimes as late as June. The drawback to early planting is that shoots of *Zostera* will not yet have flowered. Planting flowering shoots of this species will potentially add seed stock, but because the shoots die after flowering very little vegetative spreading will result from planting flowering shoots. Thus, as much as 30-40 percent more plants might need to be installed when planting early in the year to make up for this loss, unless flowers are reliably culled from plantings.

Ice shearing is a significant problem in many locales, depending, of course, upon the severity of the winter (*sensu* Robertson and Mann 1984 and F. Short, Jackson Estuarine Lab., Durham, NH, pers. com.). Coarse, cobble sediment can become especially destructive to planted seagrass when it moves during storm events. Grazing by waterfowl (Thayer et al. 1984) and green crab, horseshoe crabs, and various fishes are significant sources of bioturbation (Nereid worms have also been reported to injure plantings; F. Short, Jackson Estuarine Lab., Durham, NH, pers. com.). Also, wide tidal ranges, up to several meters, will force planting to either be done in nar-

row low-tide windows of opportunity or will require divers. In steep catchments, periodic rainfall events can become especially concentrated, rapidly driving salinities down and elevating turbidity. These aperiodic events, as with storms, may be worthy of attention when selecting sites.

Mid–Atlantic Region — Delaware through North Carolina: known species present = *Halodule wrightii*, *Ruppia maritima* and *Zostera marina*.

Planting strategies differ markedly for the species in this region. *Halodule* is a subtropical species, and like the temperate *Zostera*, is at the edge of its distribution in this region (i.e., the northern and southern limits of these two species overlap in North Carolina). For *Zostera* planting may be done from April through November, although the farther south one goes in this region, planting later in the year (September-November) gets plants into the bottom for the longest time possible before the next period of low growth (heat stress in July-August). Spring plantings of *Zostera* would still have the flowering problem described for the Northeast Region. Fall planting is also the best strategy for *Zostera* in the Chesapeake Bay (Moore and Orth 1982). For *Halodule* spring plantings are best. *Ruppia* is probably best planted in early spring as well (Bird et al. 1994). Sources of bioturbation are much the same as elsewhere in the country — rays, crabs, and horseshoe crabs.

Gulf of Mexico and the Florida East Coast — Mexico to Cape Sable and north of Jupiter Inlet to Cape Canaveral: known species present = *Halodule wrightii*, *Halophila decipiens*, *Halophila engelmanni*, *Halophila johnsonni*, *Ruppia maritima*, *Syringodium filiforme*, and *Thalassia testudinum*.

Of the seagrass species in this region, the three most commonly used species (*Halodule wrightii*, *Syringodium filiforme*, and *Thalassia testudinum*) have very different intrinsic coverage rates (Fonseca et al. 1987c). Planting should be done in the spring although plantings will survive (but spreading at lower annual rates) if initiated at other times of the year. Fonseca et al. (1994), however, found that a fall planting was more successful in high bioturbation areas (no cages used) because it apparently avoided the peak of the yearly bioturbation activity. Bioturbation has been reported by urchins, sand dollars, rays and crabs.

Coverage rates of the common species are: *Halodule wrightii* > *Syringodium filiforme* > *Thalassia testudinum*. Any of these species can be planted alone, but *H. wrightii* is considered a pioneering species and should be used to quickly establish cover. This may also be planted in alternating rows with the other species. Although *T. testudinum* may be planted alone, its very slow population growth and coverage rates under

transplant conditions make it susceptible to interim erosion. The prolonged lack of cover would also likely extend the period of interim loss of fishery resources. If turtle grass is the target species, it should be added once faster-growing species (e.g., *H. wrightii*) have stabilized the bottom.

Ruppia maritima performs much as shoalgrass when transplanted (Stout and Heck 1991, Durako et al. 1993). Its high density of rhizome apicals allows the same planting techniques to be employed as used with shoalgrass (Stout and Heck 1991). In some areas widgeon grass has reportedly been pinned to the bottom in mats, after being intertwined in a biodegradable mat or allowed to grow over mats placed in natural beds after which the mats and intertwined shoots are removed for planting elsewhere. Durako et al. (1993a) used cotton mesh bags with *Ruppia* fragments and a small stone inside for planting; bags were thrown overboard and allowed to root on the bottom by growing through the cotton bag.

The *Halophila* species (paddle grass and star grass) are extremely fragile, but can significantly reduce currents and wave scour (Fonseca 1989b). Because of their growth strategy, with only 3 or 4 leaf pairs on a rhizome in close proximity to the rhizome apical, these species would likely be suitable for transplanting using the peat pot method described in Chapter 3, although we have not tested this method at depths > 1-2 m. We have successfully transplanted *H. decipiens* bare root sprigs in 15 m of water using 60 lb. test wire fishing leader bent into a U-shape as a staple to hold the plants to the bottom until they rooted. While few cases of *Halophila* spp. transplanting have been documented, their pioneering growth strategy and small size make them likely candidates for effective use in planting projects.

South Florida and the Caribbean — South of Jupiter Inlet to Cape Sable and Puerto Rico and the U.S. Virgin Islands: known species present = *Halodule wrightii*, *Halophila decipiens*, *Halophila engelmanni*, *Halophila johnsonni*, *Ruppia maritima*, *Syringodium filiforme*, and *Thalassia testudinum*.

The same guidance should be used here as for the above section on the Gulf of Mexico and Florida east coast. The only difference is that planting can be performed at any time of the year with little difference in expected response.

Conterminous West Coast — California to Washington: known species present = *Phyllospadix scouleri*, *Phyllospadix serralatus*, *Phyllospadix torreyi*, *Ruppia maritima*, *Zostera japonica*, *Zostera marina*, and potentially, *Zostera asiatica*.

Planting should be performed during the springtime (April, May, early June). Some specimens can be very large and careful handling is required. Planting of *Phyllospadix* spp. has been practiced very little, and we can offer little guidance except to review Phillips et al.'s (1992) report (also see Chapter 1, "Comparative Analysis of Seagrass Planting Efforts"). Cold water may, as in the Northeast, contribute to high planting costs. In some areas such as San Francisco Bay, there are extensive annual populations of *Zostera* which are problematic for transplanting. Transplanting of annual *Zostera* means there will be little vegetative spread (the shoots die after flowering). Seed deposition is the only mechanism that would sustain the transplant and this is very risky unless a site is extremely quiescent; even then seed predation remains a potential problem. Experimentation with seeding techniques would be appropriate in such settings. Vertical zonation of *Phyllospadix* spp. in the rocky intertidal must be recognized and matched with local distributions. Bioturbation sources include crabs, rays, some of these fishes, and sand dollars. Bioturbation in the rocky intertidal has not, to our knowledge, been documented for planted seagrass.

Alaska — known species present = *Zostera marina* and assumed some *Phyllospadix* spp.

Little is known about planting requirements here except that spring plantings are logically better and that cold water and ice shearing may be particularly problematic.

Hawaii and Pacific Territories — known species present = *Halophila hawaiiana*, *Halophila minor*, and *Halophila ovalis*.

As with Alaska, little is known here but we are aware of no reports of seagrass planting in this region. Planting guidance must be extrapolated from elsewhere.